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PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACTS OF INDIGENOUS MEDICINAL PLANT *SMILAX CHINA*

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ABSTRACT

Smilax china L. is an indigenous medicinal plant widely recognized for its therapeutic value in traditional systems of medicine across Asia. The plant has long been used for the treatment of inflammatory disorders, skin diseases, rheumatism, urinary tract infections, diabetes, and various microbial infections owing to its rich phytochemical composition. The growing interest in plant-derived bioactive compounds has encouraged extensive phytochemical investigations to identify secondary metabolites that may contribute to the pharmacological properties of medicinal plants. Methanol is considered one of the most effective extraction solvents because of its ability to dissolve a wide range of polar and moderately polar phytoconstituents, making it suitable for qualitative phytochemical screening. Therefore, the present study was undertaken to qualitatively evaluate the phytochemical constituents present in the methanolic extract of the indigenous medicinal plant *Smilax china*. The methanolic extract of *Smilax china* was subjected to standard qualitative phytochemical screening using established chemical tests for the detection of major classes of secondary metabolites. Alkaloids were detected using Mayer's, Wagner's,

Dragendorff's, and Hager's tests. Carbohydrates were evaluated by Molisch's, Benedict's, and Fehling's tests, while glycosides were identified using Modified Borntrager's and Legal's tests. Saponins were determined by Froth and Foam tests, phytosterols by Salkowski's and Liebermann-Burchard tests, phenolic compounds by the Ferric chloride test, tannins by the Gelatin test, flavonoids by Alkaline reagent and Lead acetate tests, and proteins and amino acids by Xanthoproteic and Ninhydrin tests. The qualitative analysis demonstrated the presence of several important bioactive constituents in the methanolic extract of *Smilax china*. All alkaloid tests produced positive results, confirming the occurrence of alkaloids in the extract. Similarly, carbohydrates were positively detected by Molisch's, Benedict's, and Fehling's tests. Glycosides were confirmed by both Modified Borntrager's and Legal's tests. The Froth and Foam tests indicated the presence of saponins, while phytosterols were confirmed by positive Salkowski's and Liebermann-Burchard reactions. The Ferric chloride test established the presence of phenolic compounds, whereas flavonoids were successfully detected by both Alkaline reagent and Lead acetate tests. In contrast, tannins were not detected by the Gelatin test, and proteins and amino acids were absent according to both Xanthoproteic and Ninhydrin tests. The phytochemical profile obtained in this investigation agrees with previous reports indicating that *Smilax china* is particularly rich in steroidal saponins, flavonoids, phenolic compounds, glycosides, and alkaloids, which collectively contribute to its wide range of biological activities. Recent reviews have reported that more than 130 chemical constituents have been identified from *Smilax china*, with steroidal saponins and flavonoids representing the predominant groups responsible for antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anticancer, antihyperuricemic, and wound-healing activities. Likewise, methanolic extracts have previously been shown to contain numerous phytochemicals with considerable antioxidant potential, supporting the effectiveness of methanol as an extraction solvent. The presence of alkaloids, flavonoids, phenolic compounds, saponins, phytosterols, carbohydrates, and glycosides suggests that the methanolic extract of *Smilax china* possesses significant therapeutic potential. Many of these phytochemical groups are known to exhibit antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, hepatoprotective, and anticancer properties, either individually or through synergistic interactions. The absence of tannins and proteins in the present study may be attributed to differences in plant origin, harvesting season, extraction procedure, environmental conditions, or genetic variability, as phytochemical composition is known to vary among geographical regions and extraction methods. In conclusion, the present investigation confirms that the methanolic extract of *Smilax china* contains diverse classes of

biologically active phytochemicals that may account for its traditional medicinal applications. The findings provide scientific evidence supporting the ethnomedicinal importance of this indigenous plant and establish a preliminary phytochemical basis for future pharmacological investigations. Further studies involving quantitative phytochemical estimation, chromatographic characterization, toxicity evaluation, and biological activity-guided isolation of active constituents are recommended to identify specific bioactive compounds and facilitate the development of novel plant-based therapeutic agents.

KEYWORDS: *Smilax china*; Methanolic extract; Phytochemical screening; Indigenous medicinal plant; Alkaloids; Flavonoids; Phenolic compounds; Saponins; Glycosides; Phytosterols.

1.0. INTRODUCTION AND BACKGROUND

1.1. Background

Smilax china L., referred to ‘Ba Qia’ or ‘Jin Gang Teng’ in China (Wu et al., 2010). Tibbi name of this plant is Chobchini. It is also called china root. It is a deciduous climber, native to China and Japan. Stems are unarmed and sparsely prickled; leaves are rounded, or wedge-shaped (Zainab et al., 2019). Smilax (Liliaceae) includes 350 species and is widely distributed in tropical and temperate regions throughout the world, especially in East Asia and North America (Shao et al., 2007). Many of them have been long used as medicinal herbs, especially as traditional Chinese medicines (TCM) in China (Abdala et al., 2008). As one of the most popular and important TCM in its genus, *Smilax china L*, which is also a food in Chongqing, China (Meng and Bai, 2003). Tuber root of Smilax chinensis has medicinal activity. It is used to treat syphilis, gonorrhea, swelling, abscess, and boils. It is widespread in tropical forests in India (Krishnaraju et al., 2006). Smilax chinensis is prescribed in the management of gouty arthritis (Akram et al., 2014). Smilax chinensis is also prescribed in sexual disorders, especially for chronic pelvic inflammation (Ooi et al., 2004). This plant is found in Afghanistan, Andhra Pradesh, India, Karnataka, Pakistan, Tamil Nadu, and the United Kingdom. Smilax chinensis is often used as a blood purifier especially when atrabilious malhumours is in excess and disorders such as chronic obstructions and irritation to the kidneys or bladder. It has a specific use in diseases of spasmodic origin. In combination with other useful herbs, it is administered orally to relieve chronic nervous disorders. Smilax chinensis has been used for centuries to treat various diseases. Several chemical constituents of Smilax chinensis have been isolated (Wu et al., 2010). *S. china* consists of fat, saponins,

glucosides, gum, starch, flavonoids, tannins and alkaloids (Saravanakumar et al., 2014). Feng et al., (2003) showed that 5 phenyl compounds were isolated from the roots of *S. china* and they are dihydrokaempfero l (1): 3,5,4/ -trihydroxystilbene (2): 3,5,2/,4/ -tetrahydroxystilbene (3): dihydrokaempferol 3-O-α-L-rhamnoside (engeletin, 4): and quercetin 4/ -O-βD-glucoside (5). Shao et al., (2009) found that seven flavonoids and four stilbenes were isolated and identified as dihydrokaempferol-5-O-β-D-glucoside (I): engeletin (II): isoengeletin (III): dihydroquercetin-3-O-glycoside (IV): 3, 5, 7, 3/, 5/ -pentahydroxy-flavanonol (V): astilbin (VI): quercetin-3 / -O-glycoside (VII): piceid (VIII): scirpusin A (IX): resveratrol (X): and oxyresveratrol (XI). Shao et al. (2007) reported that the six major active constituents in *S. china* are (1) Taxifolin-3-O-glycoside; (2) piceid; (3) oxyresveratrol; (4) engeletin; (5) resveratrol; (6) scirpusin A. *S. china L*. known as Jin Gang Ten, has been widely used as a traditional herbal medicine for the treatment of gout, rheumatoid arthritis and other diseases for a long time in China (Chen et al., 2011). Shu et al., (2004) confirmed that the tuber of *S. china L*. has anti-inflammatory, anticancer, and anticoagulation activities. In Chinese medicine, it has been extensively used for clinical treatment of syphilis, acute bacillary dysentery acute, chronic nephritis and antitumor (Chen et al., 2002). The rhozimes of *S. china* is commonly used as herbal materials in traditional Chinese medicine (Liang et al., 2016). Park et al., (2014) concluded that *S. china* methanol extract (SCME) has active compounds which have anti-obesity activities. Vijayalakshmi et al., (2013) reported that the ethyl acetate fraction of *S. china* rhizome showed maximum antipsoriatic activity. Chen et al., (2011) concluded that *S. china L*. exhibits anti-hyperuricemic and nephroprotective activity in hyperuricemic animals. Jeong et al., (2013) reported that *S. china* has antimicrobial, antimutagenic, antioxidant, anti-inflammatory, anti-cancer and neuroprotective effects. Shim (2012) also recognized *S. china* has a good source of natural antioxidant. Raju et al. (2012) also showed that *S. china* is an anti-diabetic plant which is responsible for the hypoglycemic activities. Bhati et al., (2011) reported that the hydroalcoholic and aqueous fractions exhibited anti-diabetic activity in rats with alloxan induced diabetes. Seo et al., (2012) indicated that *S. china* L. possesses antioxidant and antimicrobial substances, and suggested that the ethanol extract can be applied into food and cosmetic industry. Wu et al., (2010) showed that polyphenols are the active components of *S. china L*. responsible for the anti-breast tumor cell activities. Saraswathi and Nithya (2010) suggested that the hypoglycemic and hypolipidemic property of *S. china* could be useful for the treatment of diabetes. Sarvana and Felicia (2015) also claimed that *S. china* extracts have antioxidant activity which can be used to treat various diseases. Shu et al. (2006) stated that ethyl acetate extract of *S. china*

possesses remarkable anti-inflammatory effects on acute inflammation, and also displays anti-inflammatory effects on the chronic inflammation at a certain extent. Pan et al. (2014) concluded that water extraction from *S. china* (WESC) suppressed fat accumulation and decreased the weight gain in mice, which was mainly due to increase of the activity of fat oxidation enzyme in liver, promotion of the fatty acid β-oxidation. Lee et al., (2016) suggested that the extract from *S. china L.* has great potential as a cosmetic ingredient with whitening effects. Vijayalakshmi et al. (2012) have found the flavonoid quercetin in *S. china* and they have stated that it is promising for further investigations to prove its anti-psoriatic activity. Cong et al., (2016) noted that those patients who received Azithromycin therapy added with *S. china* capsules concurrently could significantly improve levels of lymphocyte subsets, cytokines and hemorheology index. Yang et al., (2019) stated that *S. china L.* ethanol extract (SCLE) could lead to a decrease in body weight gain and fat mass by inhibiting the lipid synthesis and promoting lipolysis and β-oxidation in high-fat diet (HFD) fed mice. Pharmacological studies have also suggested that *S. china* has a neuroprotective effect (Ban et al., 2006). Lee et al., (2018) demonstrated the potent therapeutic efficacy of *S. china L.*, and its potential use as a cost-effective natural alternative medicine against type 2 diabetes and its complications. *S. china L.* known as China root has been used for thousand years in numerous tribal and folk medicine. The plant is native to China, Korea, Taiwan, Japan, Philippines, Vietnam, Thailand, Myanmar and Assam. *S. china* consists of fat, saponins, glucosides, gum, starch, flavonoids, tannins and alkaloids. The rhizomes are bitter, acrid, thermogenic, anodyne, anti-inflammatory, digestive, laxative, depurative, diuretic, febrifuge and tonic. It is used in dyspepsia, flatulence, colic, constipation, helminthiasis, skin diseases, leprosy and psoriasis, syphilis, strangury, seminal weakness, general debility, detoxifies organs, cleanses blood, aids absorption and kills bacteria; it is also used for fever, epilepsy, insanity, neuralgia and stimulates digestion, increases urination, protects liver and promotes perspiration. In Chinese medicinal science, it has been used for clinical treatment of syphilis, acute bacillary dysentery acute, chronic nephritis and antitumor. On the basis of scientific literatures, *S. china L.* demonstrates important and promising health benefits. In general, treatment with natural and traditional medicine, especially *S. china L* is recommended (Shahrajabian et al., 2019).

1.2. Aim & objective of my study

1.2.1. General Objective

To study the phytochemical screening of ***Smilax china*** Roots.

1.2.2. Specific Objective

- Qualitative analysis of *Smilax china* roots for phytochemicals from methanolic extracts.
- Screening for alkaloids, carbohydrates, glycosides, phytosterols, phenol, saponins, tannins, flavonoids, proteins, and amino acids from *Smilax china* roots.

2.0. Plant profile of *Smilax china*

2.1. Scientific classification of *Smilax china:* (CABI, 2019)

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Monocotyledonae

Order: Dioscoreales

Family: Smilacaceae

Genus: Smilax

Species: *Smilax china*

2.2. Vernicular names of *Smilax china*

Unani: Kasab al seeni

Bangla: Chopcheenini

Hindi: Chopcheenee

Malayalam: China pairu

Tamil: Parinankipatte

Telugu: Galichekka

Marathi: Chopcheenee

English: China Root

Latin: *Smilax china*

Urdu: Chop chini

Arabic name: Ashusseenee, khushabusseenee. (Tabassum et al., 2020).

2.3. Common Name of *Smilax china*

China Root, Chopcheenee (Sabarisenthil et al., 2017).

2.4. Morphology of *Smilax china*:

Smilax china is a woody vine armed with small thorns all over the stem. Rhizomes are long, thick and grey colored. Leaves are simple, alternate, elliptically oblong to subrounded, 5 to 8 centimeters long, 2.5 to 4 centimeters wide; those toward the end of the branches are much smaller and veined. Petioles are about 7 millimeters long, with adnate spiculate stipules which frequently are extended into tendrils. Inflorescence arises from the upper leaf axils, 3 to 5 centimeters long. Flowers are white to yellowish-green, their pedicels subtended by bracteoles, umbellate. Berries are globose, reddish when ripe (Tabassum et al., 2020).

2.5. Habit and habitat of *Smilax china*

Smilax china L., a member of the Smilacaceae family, is widely distributed worldwide in tropical and temperate regions, especially in China, Korea, and some Southeast Asian countries (Lee et al., 2017). *Smilax china* is geographically distributed in China, Nepal, Pakistan, Mexico, Viet Nam, India, Cambodia, Thailand, Bangladesh, Belgium, Lao People's Democratic Republic, Brazil, Myanmar, Australia, Malaysia, Japan, and the United States of America. According to the database of the Global Biodiversity Information Facility (https://www.gbif.org/) and consultation of Flora of countries, Asia, including China (Taiwan, Hong Kong, and Macao, etc): harbors the most significant proportion of *Smilax china* producing sites. In Thailand, traditional practitioners have widely used it to treat cancer, AIDS, and other infectious diseases (Tewtrakul et al., 2006).

2.6. Microscopy of *Smilax china*

Transverse section of rhizome shows on its outer edge, several layer of large, tangentially elongate, thick-walled cells with large empty space which at times are filled with brown coloring matter, followed by a layer or two of bigger cells containing bundle of calcium oxalate raphides. Below this, two three layers of tangentially elongated and rectangular cells followed by a few layers of thin walled closely pitted cells. A wide zone of large thick walled and finely pitted parenchymatous ground tissue is traversed by a few obliquely cut vascular bundles. Centre core has numerous closely arranged bid conjoint, collateral and close fibre vascular bindles which are variously oriented. There is no distinct endodermis delimiting the outer ground tissue from the inner one. In the macerated material large, small and more or less isodiametric parenchymatous cells are found, large cells measure 557-813, to 976

microns in length. And 208- 272 – 700 microns in width, smaller cells measure 184x128 microns. Vascular bundles consist of 3-7 vessels, tracheid, sieve tubes and companion cells, vessels mostly show reticulate type of thickening and measure 816-2000-3200 microns and 51-125-155 microns in width some vessels are unusually long and forked. The vessels bear elongated slit like bordered pits. Tracheid is few and elongated slit or oval bordered pits. Folium consist only sieve tubes and companion cells. Encircling the folium are groups of thick walled stratified and closely pitted fibres which have big lumen. Some of these fibres are separated. A few isolated parenchymatic cells of the ground tissue contain red granular colouring matter. All parenchymatous cells contain simple and compound spherical, cup shaped, biconvex and polygonal starch grain with a central hilum which may be a point, slit or radiating fissure. Compound grains of two to more than fifty components with flat contact surfaces are also present (Jaunpuri, 1997).

2.7. Physical description of *Smilax china*

Smilax china L is a deciduous climber, native to China and Japan. Stems are unarmed and sparsely prickled; leaves are rounded, or wedge-shaped (Zainab et al., 2019). Smilax (Liliaceae) includes 350 species and is widely distributed in tropical and temperate regions throughout the world, especially in East Asia and North America (Shao et al., 2007). Many of them have been long used as medicinal herbs, especially as traditional Chinese medicines (TCM) in China (Abdala et al., 2008).

2.8. Description of of *Smilax china* in Unani Literature

A climbing thorny shrub with sparsely prickled and unarmed stems, branchless slender, smooth and terete, leaves elliptic, unneate or acuminate, petioles 13-17mm narrowly sitting unarmed, umbels sub sessile, many flowered, penduncle abractiate, pedicles 6-8mm, bracteoles tabulate, flowers very small, white buds depressed, globes, deeply lobed from groove on the back of the obovatecoriasius sepals, petals, minute, stamen very short, staminoles in female flowers and this plant occurs throughout the year (Kabeeruddin, 1930).

2.9. Botanical description of *Smilax china*

Sarsaparillang-china is a woody vine armed with small thorns all over the stem. Rhizomes are long, thick and grey colored. Leaves are simple, alternate, elliptically oblong to subrounded, 5 to 8 centimeters long, 2.5 to 4 centimeters wide; those toward the end of the branches are much smaller and veined. Petioles are about 7 millimeters long, with adnate spiculate stipules which frequently are extended into tendrils. Inflorescence arises from the upper leaf axils, 3

to 5 centimeters long. Flowers are white to yellowish-green, their pedicels subtended by bracteoles, umbellate. Berries are globose, reddish when ripe (Kabeeruddin, 1930).

2.10. Procedures and time of collection of *Smilax china*

Tuberous root and rhizomes of the plant are carefully dug up and cut of near the stock which is covered up again with the soil. Harvested roots are washed well, peeled and trimmed to small pieces, dried in sun and tied up in bundles (Tabassum et al., 2020).

2.11. Preservation and storage of *Smilax china*

The dried drug obtained is kept in air tight bottles in cool and dried places. The medicinal effectiveness of drug last up to ten years (Jaunpuri, 1997).

Figure-1: Root of *Smilax china.*

Figure-2: Leaf and flowers of *Smilax china.*

Figure-3: Fruit of *Smilax china.*

2.12. Parts use of *Smilax china*

Root, leaves and fruit (Tabassum et al., 2020).

2.13. Mizaj of *Smilax china*

Murakkab-ul-Quwa, (Ibn Rushd, 1980). Hot 1º dry1º (Kabeeruddin, 1930).

2.14. Taste of *Smilax china*

Slightly irritating. (Tabassum et al., 2020).

2.15. Substitute of *Smilax china*

Ushba maghrabi (Smilex aristolochepholia miller) (Nafis, 1935).

2.16. Important formulation of *Smilax china*

Majun-e-Chobchini (Tabassum et al., 2020).

2.17. Method of processing of *Smilax china*

As the drug is free from any toxic side effects, it is used directly either in form of powder alone or in combination with other drugs (Kabeeruddin, 1930).

2.18. Action of *Smilax china*

Mulattif (demulcent): muhallil (Anti-inflamatory) muarriq (diaphoretic): muharik (stimulant): muaddil (alterative): musaffi Khoon (blood purifier): muqawwi azaeraeesa (visceral tonic): muqawwi bah (aphrodisiac): musakkin (analgesic) munawwim (sedative): and mudirr baul (diuretics): Hazim (digestive): Mushil (Purgative) (Ibn Rushd, 1980).

2.19. Therapeutic uses of *Smilax china* in unani medicine

The drug is used in aathishak (syphilis): juzham (leprosy): fasad dam (impurity in blood): amraze asab (nervous disorder): and waja-ul-mafasil (rheumatism): amraze kuliya-wa-masana (kidney and bladder disease) istirkha (paralysis): suda (headache): Constipation, skin disease, Parkinsonism, ulcers (Nafis, 1935) (Kabeeruddin, 1930).

2.20. Correctives (Musleh)

Anar (Saag and choi, 2006).

2.21. Dose of *Smilax china*

6 gm Powder (Tabassum et al., 2020).

2.22. Traditional uses of *Smilax china*

S. china L. known as Jin Gang Ten, has been widely used as a traditional herbal medicine for the treatment of gout, rheumatoid arthritis and other diseases for a long time in China (Chen et al., 2011). Shu et al., (2004) confirmed that the tuber of *S. china L.* has anti-inflammatory, anticancer, and anticoagulation activities. In Chinese medicine, it has been extensively used for clinical treatment of syphilis, acute bacillary dysentery acute, chronic nephritis and antitumor (Chen et al., 2002). The rhozimes of *S. china* is commonly used as herbal materials

in traditional Chinese medicine (Liang et al., 2016). Root is alternative, anti-scrophulatic, carminative, depurative, diaphoretic, diuretic and tonic. It is useful when taken internally in the treatment of old syphilitic cases and it also used for certain skin diseases, including psoriasis, rheumatoid, arthritis, gout, enteritis, urinary tract infections, skin ulcers, etc. Large doses can cause nausea and vomiting, which is appreciated in weakened and depraved conditions due to a poisoned state of the blood. Smilax is helpful in improving muscle mass and body strength. It is used as a tonic for male sexual energy. Smilax has a special property as it acts against the problems caused due to malnourishment of Dhatus such as poor immunity and weakness. Decoction of roots and rhizomes used as depurative in cases of herpetism and syphilis. It is Sudorific and demulcent, used in rheumatism. It is used for various skin diseases. It is used as a depurative, diaphoretic, stimulant, alterative, antisyphilitic and asphrodisiac It is used as alterative in old syphilitic cases and in chronic rheumatism. In TCM, used as diuretic and for treatment of rheumatic arthritic conditions; also used for detoxification, treatment of gout, tumors and lumbago. It is used for syphilis, skin disease, epilepsy, insanity, flatulence, dyspepsia, constipation, fever, neuralgia, rheumatism, gout and general debility in Ayurveda, Siddha and Unani medical system. It is used as a remedy for inflammatory disease and ischuria. Rhizome is made into a paste and applied to painful swellings. It has also been supported in the treatment of leprosy, scrofula and many skin infections developing into ulcers. Roots have been used to treat abscesses, pyoderma and burns. It was one of the drugs used in the treatment of acute appendicitis, taeniasis and constipation. Roots have been used to treat cases of paralysis and sciatica. It is used to treat urinary tract infection, stone and ulcers of the bladder and even chyluria by the physicians. It is also used to treat fever and other inflammatory conditions associated with fever like acute lymphadenitis. It helps in relieving strangury and also seminal weakness (Shahrajabian et al., 2019).

2.23. Medicinal uses of *Smilax china*

Park et al., (2014) concluded that *S. china* methanol extract (SCME) has active compounds which have anti-obesity activities. Vijayalakshmi et al., (2013) reported that the ethyl acetate fraction of *S. china* rhizome showed maximum antipsoriatic activity. Chen et al. (2011) concluded that *S. china L*. exhibits anti-hyperuricemic and nephroprotective activity in hyperuricemic animals. Jeong et al., (2013) reported that *S. china* has antimicrobial, antimutagenic, antioxidant, anti-inflammatory, anti-cancer and neuroprotective effects. Shim., (2012) also recognized *S. china* has a good source of natural antioxidant. Raju et al.,

(2012) also showed that *S. china* is an anti-diabetic plant which is responsible for the hypoglycemic activities. Bhati et al., (2011) reported that the hydroalcoholic and aqueous fractions exhibited anti-diabetic activity in rats with alloxan-induced diabetes. Seo et al., (2012) indicated that S. china L. possesses antioxidant and antimicrobial substances, and suggested that the ethanol extract can be applied into food and cosmetic industry. Wu et al., (2010) showed that polyphenols are the active components of *S. china L*. responsible for the anti-breast tumor cell activities. Saraswathi and Nithya (2010) suggested that the hypoglycemic and hypolipidemic property of *S. china* could be useful for the treatment of diabetes and *S. china* extracts have antioxidant activity which can be used to treat various diseases. Shu et al. (2006) stated that ethyl acetate extract of *S. china* possesses remarkable anti-inflammatory effects on acute inflammation, and also displays anti-inflammatory effects on the chronic inflammation at a certain extent. Pan et al. (2014) concluded that water extraction from *S. china* (WESC) suppressed fat accumulation and decreased the weight gain in mice, which was mainly due to increase of the activity of fat oxidation enzyme in liver, promotion of the fatty acid β-oxidation. Lee et al. (2016) suggested that the extract from *S. china L*. has great potential as a cosmetic ingredient with whitening effects. Vijayalakshmi et al. (2012) have found the flavonoid quercetin in *S. china* and they have stated that it is promising for further investigations to prove its anti-psoriatic activity. Cong et al. (2016) noted that those patients who received Azithromycin therapy added with *S. china* capsules concurrently could significantly improve levels of lymphocyte subsets, cytokines and hemorheology index. Yang et al. (2019) stated that *S. china L*. ethanol extract (SCLE) could lead to a decrease in body weight gain and fat mass by inhibiting the lipid synthesis and promoting lipolysis and β-oxidation in high-fat diet (HFD) fed mice. Pharmacological studies have also suggested that *S. china* has a neuroprotective effect (Ban et al., 2006). Lee et al. (2018) demonstrated the potent therapeutic efficacy of *S. china L*., and its potential use as a cost-effective natural alternative medicine against type 2 diabetes and its complications. Nho et al. (2015) reported that *S. china L*. extract (SCLE) exerts an anti-metastatic effect on human breast cancer cells.

2.24. Chemical composition of *Smilax china*

Phytochemical screening of methanol extract contains saponins, sarsponin and parallin, which yield isomeric sapogenin, alkaloids, flavonoids, sasapogenin and smilogenin. It also contains sitosterol and stigmasterol in the free from and as glucosides. Root contains fat, sugar, glucoside, coloring matter, gum and starch. Root also yields smilacin, tannin, resin,

cinchonin and saponin. Dried rhizomes yield fat, sugar, glycoside, glycoside, coloring matter, saponin, tannin, cinchonin. smilacin, and starch. Leaves reported to contain rutin. Ethyl acetate fraction of an ethanol extract of rhizomes yielded seven compounds, structure of three were established as dihydrokaempferol-5-O-beta-D-glucoside, beta-sitosterol, and daucosterol. Study of leaves yielded two new flavonoids, bismilachinone and smilachinin, together with 14 known compounds. Study of rhizomes yielded 13 compounds and identified as kaemperol-7-O-beta-Dglucopyranoside (1): engeletin (2): isoengeletin (3): kaempferol (4): dihydrokaempferol (5): dihydrokaempferol-5-O-P-D-glucopyranoside (6): rutin (7): kaempferol- 5-O-beta-Dglucopyranoside (8): 3, 5, 4'-trihydroxystibene (9): vanillic acid (10): 3, 5-dimethoxy4-O-beta-Dglu-copyranosylcinnamic acid (11): beta-sitosterol (12): and beta-daucosterol (13) (Tabassum et al., 2020).

S. china consists of fat, saponins, glucosides, gum, starch, flavonoids, tannins and alkaloids (Saravanakumar et al., 2014). Feng et al. (2003) showed that 5 phenyl compounds were isolated from the roots of *S. china* and they are dihydrokaempfero l (1): 3,5,4/ -trihydroxystilbene (2): 3,5,2/ ,4/ -tetrahydroxystilbene (3): dihydrokaempferol 3-O-α-L-rhamnoside (engeletin, 4): and quercetin 4/ -O-βD-glucoside (5). Shao et al. (2009) found that seven flavonoids and four stilbenes were isolated and identified as dihydrokaempferol-5-O-β-D-glucoside (I): engeletin (II): isoengeletin (III): dihydroquercetin-3-O-glycoside (IV): 3, 5, 7, 3/, 5/ -pentahydroxy-flavanonol (V): astilbin (VI): quercetin-3 / -O-glycoside (VII): piceid (VIII): scirpusin A (IX): resveratrol (X): and oxyresveratrol (XI). Shao et al. (2007) reported that the six major active constituents in *S. china* are (1) Taxifolin-3-O-glycoside; (2) piceid; (3) oxyresveratrol; (4) engeletin; (5) resveratrol; (6) scirpusin A. Structural compounds 1 to 6 identified from *S. china*.

The phytochemicals such as spirostanol triglycoside (dioscin) and steroidal Saponin glycosides such as Diosgenin, Smilagenin and Sarsasapogenin have been reported in parts of this plant. Flavonoids, tannins and phenolic acids are polyphenolic compounds, generally found in plants, have been reported to show multiple biological effects such antipyretic and antimicrobial activities. Thus, according to Udinn et al., the presence of phenols, glycosides, tannins and flavonoids may play a crucial role for its efficacy in various activities such as antioxidant and cytotoxic activities (Uddin et al., 2015; Raúl et al., 2017; Liang et al., 2016 & Tian et al., 2017).

According to chromatographic and spectral analysis performed on parts of Smilax zeylanica contains a wide variety of phytochemicals such as Diosgenin, Smilagenin, sarsasapogenin, hydroxyltyrosol and squalene are the chemical compounds found in most of the plants of genus Smilax which is said to be responsible for various pharmacognostic activities. The preliminary phytochemical compounds present in different plant parts along with the various extracts extracted. In genus Smilax, steroidal saponins could be divided into four groups based on its structure, i.e., i) isopirostane, ii) furostane, iii) pregame, iv) cholestane (Tian et al., 2017).

The bioactive compounds such as diosgenin, dioscin and smilagenin belong to isopirostane group of steroidal saponins while sarsasapogenin belongs to Spirostane type of saponins. Both the classes of steroidal saponins are monodesmosidic glycosides, the later characterized by an axial oriented methyl or hydroxymethyl and F-ring and the former characterized by an equatorial oriented methyl or hydroxymethyl (C-27) on F-ring (Venugopalan and pernumal, 2011).

2.25. Pharmacological activities of *Smilax china*

2.25.1. Anti-inflammatory activity of *Smilax china*

The presence of Sieboldogenin in *Smilax china* shows the significant lipoxygenase inhibition (IC50: 38μM). It also exhibited significant inhibition ($p<0.05$) of carrageenaninduced hind paw edema at the doses of 10 and 50mg/kg. Computational molecular docking shows that the molecular interaction with essential amino acid residues in the catalytic site of lipoxygenase, revealing its potential binding form at molecular level (Khan et al., 2009). Study in rats of aqueous extract of tubers of *Smilax china* showed anti-nociceptive and anti-inflammatory effects. Results showed inhibition of both COX-2 activity and COX expression (Tabassum et al., 2020). Study isolated seiboldogenin from ethyl acetate fraction of the plant crude extract. Seiboldogenin showed significant lipoxygenase inhibition and significant inhibition of carrageenan-induced hind paw edema and presents as a potential new anti-inflammatory compound (Sturrock and R.D 2000).

2.25.2. Anticancer activity of *Smilax china*

The anticancer activity of eight crude extracts of *Smilax china* rhizome (SCR) against HeLa cells was assessed by MTT assay and clonogenic assay, the fraction rich in flavonoids had showngood activity against HeLa cells. A bioassay-guided separation on this extract lead to

the detection of kaempferol-7-O-β-D-glucoside (KG): which belongs to flavonoid glycoside, displayed marked anticancer activity (Li et al., 2007).

2.25.3. Antioxidant activity of *Smilax china*

The ethyl acetate fraction of *Smilax china* showed the highest antioxidant property, correlating with the high phenolic levels, particularly catechin and epicatechin (Jeong et al., 2013). In this study, a possible presence of antioxidant activity of *Smilax china* root extract was investigated. Methanol extract (Me) revealed the presence of high 1,1-diphenyl-2picrylhydrazyl (DPPH) free radical scavenging activity (IC50 7.4 µg/ml) and protective property of cell's viability. Further fractionation with various solvent extraction and assay showed high levels of DPPH free radical scavenging activity in the ethyl acetate, butanol and water extracted fractions. In addition, V79-4 cells treated with Me of *Smilax china* root induced an increase of superoxide dismutase, catalase and glutathione peroxidase activities in a dose-dependent manner between 4-100 µg/ml. These results suggest that the medicinal component of the root of *Smilax china* extracts also contains antioxidant activity (Lee et al., 2001). Various leaf extracts were evaluated for antioxidant and antimicrobial activities. The ethanol extract showed the highest DPPH, ABTS radical scavenging activity, and reducing power. All extracts inhibited the growth of Listeria monocytogenes, S. aureus, and Salmonella typhimurium. Results suggest potential use in the food and cosmetic industry. Various extracts were tested for radical scavenging and antioxidant activities. The ethyl acetate fractions showed the highest antioxidant activity, correlating with the high phenolic levels, particularly catechin and epicatechin (Tabassum et al., 2020).

A study carried out on N-nitrosodiethylamine induced hepatocarcinogenesis and benzo(a)pyrene treated Wister albino male rats showed that rats treated with the leaf extract resulted in rise in enzymatic as well as non-enzymatic antioxidants, there was also a considerable decrease in lipid peroxidation (Rajesh and Perumal, 2013).

The methanolic extract of leaves of Smilax zeylanica showed antioxidant function on diabetic rats; rats administered with leaf extract showed improved lipid peroxidase level in the liver and kidney tissues, other than that it showed decline in antioxidant enzymes, i.e., glutathione peroxidase, SOD, Catalase, glutathione reductase, non-enzymatic antioxidants and glutathione S-transferase suggesting that it has a good efficacy as an antioxidant agent. Murali et al. performed study on in vivo antioxidant activity on albino rats which showed that the roots and rhizome extract (methanolic) of Smilax zeylanica suppressed the lipid

peroxidation and increased the production of enzymatic antioxidants in rats treated with CCl4 (carbon tetrachloride). The root extract when administered in male rats induced with catalepsy by haloperidol improved TBARS production and significantly lowered the glutathione (GSH): superoxide dismutase (SOD): and catalase amount. Male rats induced with catalepsy by haloperidol when treated with root extract showed improved TBARS production and significantly lowered the glutathione (Thirugnanasampandan et al., 2009).

Divya evaluated antioxidant activity by performing peroxidase, SOD, catalase and glutathione assay on Wister albino rats; it was noted that there was a significant increase in the enzyme when animals were treated with ethanol and aqueous extract of *S. zeylanica*, also the ethanolic extract demonstrated maximum activity among all the three extracts. Other activities such as anti-arthritic as well as anti-inflammatory activity can be interdependent to each other (Divya, 2014).

2.25.4. *Smilax china* protecting induction of lipid peroxidation

The alcoholic extract of *Smilax china* protects the induction of lipid peroxidation, induced by $FeSO_4$. This may be due to chelation of iron, conversion of $Fe^{2+}$to Fe^{3+}, by increased level of reduced glutathione, or by scavenging hydroxyl, superoxide radicals, and other oxygen molecules responsible for lipid peroxidation (Triphathi and Upadhyay, 2001).

2.25.5. Testicular free radical scavenging activity *of Smilax china*

The ethanolic extract of *Smilax china* rhizomes at 100 and 200mg/kg b.w doses showed significant testicular free radical scavenging activity and restoration to normal spermatological parameters (Saraswathi et al., 2012).

2.25.6. Antidiabetic activity of *Smilax china*

The methanolic extract of *Smilax china* has significant Antidiabetic activity in Alloxan induced diabetes in rats. The maximum reduction in glucose level was seen at the dose of 400 mg/kg b.w. The antidiabetic activity may be due to promotion of insulin secretion by closure of potassium-ATP channels, membrane depolarization and stimulation of Calcium influx, an initial key step in insulin secretion **(**Sabarisenthil et al., 2017). *Smilax china* L., a native plant found in Asian countries, has several medicinal properties including antioxidant, anti-inflammatory, and anticancer effects. Although the root of the plant is commonly used as traditional herbal medicine in Korea and China, the medicinal properties of the leaves have not gained the same attention. In this study, we analyzed the antioxidant activity, αglucosidase inhibitory effect and lipid accumulation inhibition effect of *Smilax china* L. leaf

water extract (SCLE) and its solvent fractions. SCLE was fractionated by using a series of organic solvents, including ethylacetate (EA) and nbutanol (BuOH). The EA fraction had the highest total polyphenol content (440.20 ± 12.67 mg GAE/g) and total flavonoid content (215.14 ± 24.83 mg QE/g). The radical scavenging activity IC50 values of the EA fraction for 2,2-diphenyl-1picrylhydrazyl (DPPH) and 2,2-azino-bis-(3ethylbenzthiazoline)-6-sulfonic acid (ABTS) were 0.022 mg/mL and 0.13 mg/mL, respectively. Further, SOD-like activity and reducing power values of the EA fraction were higher than those of the other fractions. However, both the αglucosidase and lipid accumulation inhibition assays showed that the BuOH fraction (83.35 ± 4.18% at 1 mg/mL) and water extract (11.27 ± 2.67%) were more effective than the EA fraction (64.13 ± 6.35%, and 45.66 ± 7.20%). These results provide new insights into the potential antidiabetic and anti-obesity effects of *Smilax china L*. leaf (Sabarisenthil et al., 2017). Study of leaves isolated two new flavonoids, bismilachinone and smilachinin, together with 14 known compounds, from the leaves of *Smilax china*. Compounds 4, 7, and 10 showed moderate DPP-IV inhibitory activities, while compounds 3, 4, 6, 11, 12, and 16 showed strong PTP1B inhibitory activities. Compounds 2-7, 11, 12, 15, and 16 showed α-glucosidase inhibitory activities. Results show the leaves of *Smilax china* may contain antidiabetic compounds (Tabassum et al., 2020).

2.25.7. Anti-obesity activity of *Smilax china*

In this study, the anti-obesity activity of *Smilax china* methanol extract (SCME) was evaluated using a pancreatic lipase enzyme inhibition assay, and a cell culture model system. Results indicated that, SCME effectively inhibited pancreatic lipase enzyme activity in a dosedependent manner. Furthermore, SCME significantly suppressed insulin, dexamethasone, 3-isobutyl-1-methyl xanthine-induced adipocyte differentiation, lipid accumulation, and triglyceride contents on 3T3-L1 preadipocytes, in a dose-dependent manner. The anti-adipogenic effect was modulated by cytidine-cytidineadenosine-adenosine-thymidine (CCAAT)/ enhancer binding proteins (C/EBP) a, C/EBPp, and the peroxisome proliferator-activated receptor y gene and protein expressions. Moreover, SCME triggered lipolysis effects dose-dependently on adipocyte. Taken together, these results provide an important new insight into SCME, indicating that it possesses anti-obesity activity through pancreatic lipase inhibition, anti-adi-pogenic and lipolysis effects. SCME may therefore be utilized as a promising source in the field of nutraceuticals. The identification of active compounds that confer the anti-obesity activities of SCME may be a logical next step (Sabarisenthil et al., 2017). Study investigated the lipolytic actions of a water-soluble fraction

of *Smilax china* leaf ethanol extract in 3T3-L1 adipocytes. Results showed potential antiobesity activity, which may be due, at least in part, to stimulation of cAMP-PKA-HSL signalling. The wsSCLE-stimulated lipolysis induced by signalling is mediated by activation of ß-adrenergic receptor (Tabassum et al., 2020).

2.25.8. Anti-metastatic activity on Human breast cancer cells

This study was to investigate the effect of a SCL ethanol extract (SCLE) on the proliferation and migration of MDA-MB-231 human breast cancer cells, as well as the expression of urokinase plasminogen activator (uPA): uPA receptor (uPAR) and tissue inhibitors of metalloproteinases (TIMPs). Cell proliferation was assessed using the Cell Counting Kit-8 and cell migration was determined by wound healing assay. Quantitative polymerase chain reaction was performed to quantify the mRNA levels of uPA, uPAR and TIMPs. SCLE markedly inhibited the proliferation and migration of MDA-MB-231 cells, and reduced the mRNA levels of the extracellular matrix (ECM) degradation-associated molecules uPA, uPAR. By contrast, SCLE significantly increased the mRNA levels of TIMP1 and TIMP2. These findings show that SCLE exerts an anti-metastatic effect on human breast cancer cells, which may involve the modulation of ECM degradation (Nho et al., 2015).

2.25.9. Hepatoprotective activity of *Smilax china*

In this study Smilax chinensis extract affords hepato protection against carbon tetrachloride through cell membrane stabilization and hepatic cell regeneration (Venkidesh et al., 2010). Study on CCl_4 induced hepatotoxicity in Wistar rats exposed the antihepatotoxic potential of leaf extract of *S. zeylanica*. Rats treated with methanolic leaf extract showed significant reduction in elevated levels of SGOT, SGPT ($p<0.01$, $p<0.05$) also regenerating hepatocytes and normalizing the fatty changes in cells indicating anti-hepatotoxic activity of extract (Murali et al., 2014). Additional study performed by Murali et al. revealed the anti-hepatotoxic activity of roots and rhizome of *S. zeylanica* in Wistar rats using paracetamol induced hepatotoxicity. The root and rhizome extract when administered to the animal showed significant reduction in the elevated alanine transaminase (ALT): aspartate transaminase (AST): alkaline phosphatase (ALP): total bilirubin and liver weight, also along with it an increase in the level of total protein and albumin when compared with the standard drug used which were proved statistically significant through Anova (Murali et al., 2012).

2.25.10. Anti-inflammatory and Analgesic activity of *Smilax china*

The ethyl acetate and methanolic extract of *Smilax china* showed dose dependent antiinflammatory activity and produced reduction in the duration of licking in the late phase in analgesic activity, which was found to be statistically significant at higher concentration in acute carrageenan induced rat paw edema model and eddy's hotplate method respectively (Reddy et al., 2010).

2.25.11. Anti-hyperuricemic activity of *Smilax china*

In this study ethylacetate fraction (250 mg/kg) of *Smilax chinensis* was exhibited stronger antihyperuricemic activity in hyperuricemic mice. Caffeic acid, resveratrol, rutin and oxyresveratrol isolated from ethylacetate fraction showed different inhibitory activities on xanthine oxidase in vitro, with the IC (50) values of 42.60, 37.53, 42.20 and 40.69μM, respectively, and exhibited competitive or mixed inhibitory actions. Moreover, it (125, 250 and 500 mg/kg) markedly reversed the serum uric acid level ($p<0.05$, $p<0.01$ and $p<0.001$, respectively): fractional excretion of urate ($p<0.05$, $p<0.01$ and $p<0.01$, respectively) and blood urea nitrogen ($p<0.05$, $p<0.01$ and $p<0.01$, respectively) to their normalstates, and prevented the renal damage against tubulointerstitial pathologies in hyperuricemic rats (Chen et al., 2011).

2.25.12. Anti-diabetic activity of the plant extracts of *Smilax china*

The hydroalcoholic and aqueous extracts of *Smilax china L*. (1 g/kg) produced a significant reduction in blood glucose levels in alloxan-induced diabetic rats. The percentage reduction in blood glucose levels in group III, group IV, and group V on day 4 and day 10 exhibited significant differences ($P<0.01$) when compared with the percentage blood glucose reduction at day 0 within the same group. Group IV and group V exhibited significant differences in reducing the blood glucose levelwhen compared with the standard control group III at $P<0.05$ confirming a more potent extract activity compared with the standard control. This significant reduction in blood glucose by *Smilax china L*. (50% ethanolic and aqueous extracts) in comparison with the standard drug (gliclazide) confirms its usefulness as a treatment for diabetes (Bhati et al., 2011). Jena et al., (2012) evaluated the antidiabetic activities of leaves in different extracts by using streptozotocin induced diabetic Wistar rats. It was observed that all the extract showed a remarkable antidiabetic activity, almost comparable to the standard drug (glibenclamide: used for the treatment of type II diabetes mellitus) at concentration of 250 mg/kg. There was a notable reduction in blood sugar level in the rats treated with *S.*

zeylanica leaf extract, among all, the ethanolic extract showed much appropriate results; it was further evaluated through statistical analysis using one way ANOVA ($p<0.05$). By increasing pancreatic secretion of insulin from beta cells of islets of Langerhans or by increasing peripheral glucose uptake can be mechanism of the plant extract for its antidiabetic activity (Jena et al., 2012). Another study performed by Rajesh and Perumal on streptozotocin induced diabetic rats revealed dose dependent hypoglycemic activity of methanolic leaf extract indicated by elevation in the insulin level in the serum of the rats administered with the leaves extracts and decrease in the level of glucose; also extract treatment showed a considerably decreased level of urea and creatinine (Rajesh et al., 2012). A recent study performed by Tyagi et al. for evaluating antidiabetic properties of roots of *S. zeylanica* in streptomycin induced diabetic rats by orally administering the extract (400 mg/kg). Methanolic extract showed exceeding effect in controlling blood sugar (108.7±6.8, 122.6±8.4 and 117.9±4.7) after 7, 14 and then 21 days respectively. Also, the histopathology examination showed that there was no destruction noted in the shapes of β-cell of islet of pancreas (Tyagi et al., 2019).

2.25.13. Endothelial Dysfunction study of *Smilax china*

This study investigated the effects of compounds isolated from 70% ethanol (EtOH) extraction of *Smilax china L*. (SCE): a plant belonging to the family Smilacaceae on nicotineinduced endothelial dysfunction (ED) in human umbilical vein endothelial cells. We isolated 10 compounds from ethyl acetate (EtOAc) fraction of 70% EtOH extract of SCE and investigated their inhibitory effect on nicotine-induced ED in endothelial cells. Kaempferol, kaempferol 7-O-α-Lrhamnopyranoside, puerarin and ferulic acid showed strong inhibition of nicotine-induced vascular cell adhesion molecule (VCAM-1) expression while kaempferol, kaempferin, and caffeic acid attenuated intercellular adhesion molecule (ICAM-1) expression. Lepidoside, caffeic acid and methylsuccinic acid caused the highest up-regulated expression of endothelial nitric oxide synthase at the protein level with caffeic acid and ferulic acid showing strong inhibitory effects on inducible nitric oxide synthase (iNOS) expression. In addition, ferulic acid and kaempferol showed inhibition against interleukin-8 (IL-8) and interleukin-1β (IL-1β) expression while ferulic acid and caffeic acid showed comparatively higher inhibition of ED associated tumor necrosis factorα (TNF-α) expression. These results show the potential of the aforementioned compounds to reverse the toxic effects of nicotine on the endothelium (Lincha et al., 2016).

2.25.14. Anti-metastatic activity on Human breast cancer cells

This study was to investigate the effect of a SCL ethanol extract (SCLE) on the proliferation and migration of MDA-MB-231 human breast cancer cells, as well as the expression of urokinase plasminogen activator (uPA): uPA receptor (uPAR) and tissue inhibitors of metalloproteinases (TIMPs). Cell proliferation was assessed using the Cell Counting Kit-8 and cell migration was determined by wound healing assay. Quantitative polymerase chain reaction was performed to quantify the mRNA levels of uPA, uPAR and TIMPs. SCLE markedly inhibited the proliferation and migration of MDA-MB-231 cells, and reduced the mRNA levels of the extracellular matrix (ECM) degradation-associated molecules uPA, uPAR. By contrast, SCLE significantly increased the mRNA levels of TIMP1 and TIMP2. These findings show that SCLE exerts an anti-metastatic effect on human breast cancer cells, which may involve the modulation of ECM degradation (Nho et al., 2015).

2.25.15. Testicular antioxidant activity and spermatological parameters

The aim of the present study was to investigate the potential benefits of ethanolic extract of *Smilax china* Linn. on testicular antioxidant activity and spermatological parameters in rats subjected to forced swimming stress. Animals of the experimental groups except vehicle control were subjected to forced swimming stress (FSS) 15 min/day for 52 days. Animals were pretreated with two doses of ethanolic extract of *Smilax china* rhizomes (100 and 200 mg/kg b.w., p.o.) for 15 days prior to the starting of FSS and were continued further for 52 days along with induction of stress. Testicular SOD, catalase and lipid peroxidation were determined. The cauda epididymis was isolated and sperms were released into saline and sperm count, viability, morphology and motility were analysed. Rats with forced swimming stress showed a significant increase in lipid peroxidation and decrease in testicular SOD, catalase, sperm count, viability and motility. Many abnormal forms of sperms were seen. Rats pretreated with ethanolic extract of *Smilax china* rhizomes at both doses significantly prevented the stress induced changes. Hence, ethanolic extract of *Smilax china* rhizomes at both doses showed good protection against testicular antioxidant activity and spermatological parameters in rats subjected to forced swimming stress (Sabarisenthil et al., 2017).

2.25.16. Nephroprotective / Anti-Hyperuricemic of *Smilax china*

An ethyl acetate fraction exhibited strong anti hyperuricemic activity. Caffeic acid, resveratrol, rutin and oxyresveratrol isolated from the EAF showed different inhibitory

effects on xanthine oxidase in vitro. The EAF also prevented renal damage against tubulointerstitial pathologies in hyperuricemic rats (Tabassum et al., 2020).

2.25.17. Anticonvulsant activity of *Smilax china*

Results of study of ethanolic extract and ethyl acetate fraction of the rhizome of *Smilax china* in mice showed it may help control petit mal and grand mal seizures (Sachs et al., 2009). A study was conducted to investigate the anticonvulsant effect of *Smilax chinensis* in mice. As a conclusion, it was found that *Smilax chinensis* has anti-convulsive efficacy and can be prescribed for the treatment of epilepsy (Tabassum et al., 2020).

2.25.18. Hypoglycemic / Hypolipidemic activity of *Smilax china*

Study evaluated the anti-diabetic effects of methanol extracts of *Smilax chinensis* in alloxan-induced diabetes rats. Results showed significant reduction of blood glucose ($p<0.01$) in a time-dependent manner. Extract treated groups also showed significant reduction ($p<0.01$) in serum levels of total cholesterol and triglycerides (Klemp et al., 1997).

2.25.19. Antipyretic Activity of *Smilax china*

Antipyretic activity of Smilax chinensis has been investigated. Pyrexia was induced by subcutaneous injection of yeast suspended in methylcellulose solution. Oral administration of Smilax chinensis reduced significantly elevated body temperature of the rat. This antipyretic activity was more than that of aspirin (a standard antipyretic product) (Seo et al., 2012).

2.25.20. Antimicrobial and antioxidant activity of *Smilax china*

Antimicrobial and antioxidant potential of Smilax chinensis extracts acquired with water, acetone, ethanol, and methanol were evaluated. Antioxidant potential was investigated by determining the reducing power, total phenol contents, ABTS radical scavenging effect, and DPPH radical scavenging activity. The ethanol extracts exhibited the highest reducing power, ABTS radical scavenging potential, and the DPPH activity. Paper disc method was used for determination of antibacterial potential against foodborne microorganisms. The growth of Salmonella Typhimurium, Staphylococcus aureus and Listeria monocytogenes was inhibited by all the extracts of Smilax chinensis, though no antibacterial potential was shown against Escherichia coli O157: H7. It is concluded that Smilax chinensis has antimicrobial and antioxidant ingredients, and recommend that ethanol extract can be used in the cosmetic and food industry (Balamurugan et al., 2015).

2.25.21. Anthelmintic activity of *Smilax china*

The anthelmintic activity of petroleum ether, benzene, chloroform and methanolic extracts of Smilax zeylanica on Indian adult earthworm (Pheretimaposthuma) has been evaluated and reported. Petroleum ether and chloroform extract showed potent anthelmentic activity than the standard drug, albendazole. The benzene extract is inactive at low concentration but have dose dependent action on paralysis and death at high dose. The methanolic extract showed dose dependent anthelmenticavtivity. Paralytic condition was achieved faster by petroleum ether extract even at 20mg/ml whereas death of test organism was early with methanolic extract. The result signifies that steroidal components would be active for achievement of paralytic condition, whereas flavanoids and polyphenolic compounds would somehow responsible for the death of Pheretima posthuma (Rajesh et al., 2010).

2.25.22. Effect of *Smilax china* on adjunctive arthritis mouse:

The study was done to evaluate the effect of Smilax chinensis on adjunctive arthritis (AA) of the mouse. Decoction inhibited adjunctive mouse arthritis, reduced thymus and spleen weights, and decreased CD4/CD8. The study shows that decoction has therapeutic effects on the regulation of cellular immunity (Vijayalakshmi et al., 2011).

2.25.23. Cytotoxic Anticancer Activity of *Smilax china*

Studies performed by Rajesh and Perumal revealed the anticancer nature of leaf extract of *Smilax zeylanica* in treating lung cancer in Swiss albino mice. The mice induced lung cancer by benzo(a)pyrene when administered with methanol extract of leaves resulted in reduction in the number of nodules in lungs along with increase in weight in the mice, thus suppressing lung cancer. They also explored the ability of *S. zeylanica* leaves extract against N-nitrosodiethylamine induced hepatocarcinogenesis in Wistar albino rats. The extracted treated rats showed notable improvement in the body weight gain (Extract 200 mg/kg: 194.2±9.61 g and extract 400 mg/kg: 210±1.82 g) and a significant reduction in relative liver weight at conc. 200 mg/kg was 2.60±0.23 and for 400 mg/kg 2.45±1.10, decreased levels in liver injury and liver cancer markers serum which was noted by a significant decrease in AST (aspartate transaminase): ALT (alanine transaminase): ALP (alkaline phosphatase) and GGT (gamma-glutamyl transferase) also in AFP (alpha-fetoprotein) and CEA (carcinoembryonic antigen) levels in serum 16.43±0.85–13.87±0.30 respectively (Rajesh and Perumal, 2013). A recent study by Malge et al. on cytotoxic activity by using Brine shrimp lethality assay and anticancer activity by liver carcinoma HepG2 cells using MTT assay. The aqueous extract of

root and rhizomes exhibited an 50% mortality rate at 84 µg.m also the value is close to value <100 µg.m which is considered "highly cytotoxic". The extract demonstrated significant in vitro anticancer activity at doses higher than 300 µg.m by inhibiting 77% HepG2 cell growth (Malge et al., 2021). By performing BSLA (brine shrimp lethality assay) and MTT cell viability assay, anticancer activity of CH3OH and Petroleum ether extract of stem was evaluated. The result disclosed that petroleum ether showed marked cytotoxicity in both the assays. Both the extracts exhibited cytotoxic effect against MCF7 (human breast cancer cell line) in MTT assay with a value of 15.49 and 19.19 µg/ml respectively (Uddin et al., 2015).

2.25.24. Analgesic Activity of *Smilax china*

Nithyamala et al., (2015) detected the analgesic properties of root of *Smilax zeylanica* using the hot plate procedure and the acetic acid mediated writhing method in albino mice. Root powder when administered orally showed greatly improved reaction time and also generated inhibitory effect depending on the doses.

Jena et al., (2011) using the Tail Immersion method on Swiss albino mice screened the analgesic activity of *Smilax zeylanica* extract and compared it with standard drug Aspirin. Treatment of mice with the petroleum ether extract, chloroform extract and methanol extract displayed good effects at a dose of 100 mg/kg at 1.5 h, which were comparable to the standard drug used. Statistically as well the values of Anova at 5% level of significance ($p<0.05$) does prove the results obtained experimentally. Overall, methanolic extract showed better efficacy as an anti-analgesic with tail flickering latency as 8.4±1.2 at 180 min (Jena et al., 2012). The ethanolic extract of leaves of *Smilax zeylanica* was evaluated by Hossain et al. using acetic acid-induced writhing test in Swiss albino mice. Administration of extract exhibited a dose dependent inhibition by 29.67% ($p<0.01$) at body weight 250 and 500 mg/kg respectively on number of writhing in mice expressing its analgesic potential (Hossain et al., 2013).

2.25.25. Anthelmintic Activity of *Smilax china*

The anthelmintic activity of leaves of *Smilax zeylanica* in petroleum ether, benzene, chloroform and methanol extract was evaluated by Rajesh et al. using adult Indian earthworm. It was observed that all the extracts were able to paralyze earthworms, but among the four extracts, petroleum ether and chloroform extract displayed more efficacies as compared to the standard anthelmintic drug Albendazole (Rajesh, 2009).

Another study was performed by Jena et al., (2011) on anthelmintic activity of leaves of *S. zeylanica* in various solvents (methanol, petroleum ether, diethyl ether and chloroform) against Pheretima posthuma (adult Indian earthworms). By considering the time taken for paralysis and death of worms, it was noted that in 100 mg/kg conc. of methanol extract, the death time was 42.13±0.9, whereas for 10 and 50 mg/kg 90.76±1.0 and 55.47±1.0 min respectively, all the extracts showed considerable activity at concentration of 10 mg/ml but at 100 mg/kg methanol extract showed more efficacy thus was comparable to standard drug piperazine citrate (Jena et al., 2011). Similarly, various leaf extracts of *S. zeylanica* were investigated for its anthelmintic activity by Qureshi et al. on adult Indian earthworm, Pheretima posthuma; the results revealed that alcoholic extract exhibited greatest activity by having death time 42.13±0.9/min, while chloroform showed the least activity 97.48±5.1/min (Qureshi et al., 2009).

2.25.26. Cytoprotective Activity of *Smilax china*

Rajesh and Perumal evaluated *Smilax zeylanica* leaves extract for its potential cytoprotective activity by performing lipid peroxidation assay, lactate dehydrogenase leakage into culture medium, catalase activity and the levels of reduced glutathione (GSH) in the cells along with inducing oxidative stress in L132 pulmonary cells and BRL 3A liver cells by cell viability assay. When the cultured cells were pre-treated with methanolic extract of leaves, it was observed that there was a considerable increase in the cell viability, decreased lipid peroxidation, LDH leakage and increased catalase and reduced glutathione content in the cell thus exhibiting cytoprotective activity. Thus, their study suggested that *Smilax zeylanica* acts as a strong cytoprotective agent against oxidative injury due to reactive oxygen species (Rajesh and Perumal, 2013).

2.25.27. Antibacterial Activity of *Smilax china*

Hossain et al., (2013) investigated anti-bacterial activity of ethanol leaves extract using disc diffusion method against a panel of gram-positive and gram-negative bacterial strain. The leaf extract results obtained showed zone of inhibition ranging from 5.39 to 9.21 mm and 9.87 to 12.55 mm; along with it, it was observed that the extract showed concentration dependent inhibition against all the pathogens among which highest inhibition was noted against Salmonella paratyphi and the least against Escherichia coli respectively. Another study by Sarbadhikary et al. (2015) assessed the potential anti-bacterial properties of ethanolic extract of leaves by performing disc diffusion assay. The extract showed inhibitory

effect against E. coli more profusely with a zone of inhibition of 10 mm while against other bacteria there was no inhibition. The methanol extracts of the fruit and leaves were subjected for antifungal analysis using agar well diffusion method on fungal organism such as Escherichia coli, Shigella flexneri and Salmonella typhimurium. Both the extracts showed significant zone of inhibition, the highest zone of inhibition noted was B. cereus whereas, it was the least in S. typhimurium (Dhanya et al., 2018).

2.25.28. Antifungal Activity

Dhanyashree et al., (2018) investigated antifungal properties of methanol extract of leaf and fruit of *Smilax zeylanica* for inhibiting seed borne fungi assessed using poisoning the medium technique on two fungal species Bipolaris sps and Aspergillus niger. Both the extracts i.e., leaf and fruit extracts demonstrated a marked inhibition of mycelial growth of the fungi more than 50%, Bipolaris sps was more susceptible as compared to A. niger.

Saha et al, (2018) while studying on various plant extracts as antifungal against fungal pathogens Curvularia eragrostidis (P. hennings) Meyer., Colletotrichum camelliae Mess, Pestalotiopsis theae (saw) stey., and Botryodiplodia theobromae Patouiilard. The ethanol and aqueous extracts of *Smilax zeylanica* showed 100% inhibition of spore germination against all the fungal pathogens.

2.25.29. Neuroprotective Activity of *Smilax china*

Senthil et al., (2018) screened the ethanolic root extract of *S. zeylanica* on neuroprotective effect by aluminium chloride induced cognition in rats. It was evaluated on the basis of five behavioral studies viz. locomotor activity, Elevated plus maze test (EPM): Y-Maze test, passive avoidance test and acetylcholinesterase activity. Oral administration of the extract increased learning and memory in the rats evaluated by the behavioural models and also suggested that the extract may act as anti-Alzheimer's effect through behavioral, cognitive and anti-AChE activities. A recent study by Yokeshwaran et al, (2020) on various extracts of *S. zeylanica* was investigated for AchE-inhibitory activity using adult male Wistar rats and adult female albino mice and Ellman's method, the animals were given doses of the extract and were observed for 14 days, at concentration of 150 mg/kg the extract exhibited inhibitory activity in AlCl3 treated rats by decrease in acetylcholine level.

2.25.30. Immunomodulatory Activity of *Smilax china*

Babu et al., (2017) investigated the immunomodulatory property of methanolic root extract by NBT reduction test. The result showed that there was increase in the neutrophils to phagocytic activity by 38 and 43% respectively at concentration 200 and 400 mg/kg compared to the control; also, there was suppression of the intercellular killing potential of phagocytes by the root extract by intercellular reduction of NBT dye.

2.25.31. Pesticidal Activity of *Smilax china*

The chloroform and methanolic extract of leaf, stem and root were studied for pesticidal activity against the adult flat grain beetle Cryptolestes psuillus through Surface Film method. Mortality rate was recorded at 24, 48 and 72 h post exposure and it was noted that methanolic (Bari et al., 2010).

2.25.32. Anti-cataleptic Activity of *Smilax china*

Anti-cataleptic activity of ethanolic dried root extract of *S. zeylanica* was screened using block method against haloperidol induced catalepsy in male Wistar rats which revealed that treatment of the extract showed a notable decrease in the scores in all the drug-treated groups as compared to the haloperidol treated group showing maximum reduction observed in animals treated with 400mg/kg of extract to its body weight (Ahemad et al., 2012).

2.25.33. Antipyretic Activity of *Smilax china*

A study performed by Jena et al. evaluated antipyretic activity of dried leaves of *S. zeylanica* in different solvent by Brewer's yeast induced pyrexia in rats. The results suggested that ethanolic extract showed good results in maintaining normal body temperature in the rats at a dose of 200 mg/kg; after 3 h of treatment, the body temperature noted was 38.68±12 which is similar to the action of the standard antipyretic drug paracetamol (Jena et al., 2012).

2.25.34. Anticonvulsant Activity

Along with anti-pyretic activity, Jena et al. also evaluated the anticonvulsant activity of dried leaves extracted by strychnine induced tonic convulsions in Swiss albino mice. Treatment of mice with the extract at dose 400 mg/kg resulted in reduced convulsions in mice after 30 min. Ethyl acetate extract showed marked activity by having 1.6±0.4 convulsion number and 100% survival rate; this was statistically determined through ANOVA $p<0.01$ and Dunne's t-test (Jena et al., 2012).

2.25.35. Thrombolytic Activity of *Smilax china*

Rahman et al. investigated extracts of different parts of *S. zeylanica* for thrombolytic activity by using clot lysis method. Among all, the methanolic and chloroform root extract showed highest activity 33.63±0.83 and 32.02±1.46% respectively.[61] Another study by Hossain et al. using petroleum ether and ethanol extract of leaves of *S. zeylanica* by clot lysis assay. Ethanol extract showed more efficacy for clot lysis (43.35%) than that of petroleum ether extract (19.59%) (Moazzem et al., 2014).

2.25.36. Antidepressant Activity of *Smilax china*

Antidepressant activity of dried roots of *S. zeylanica* was studied by Ahmed et al., (2016) by subjecting mice to forced swimming test and tail suspension test. The mice were observed; after 2 weeks of treating the mice with root extract there was significant decrease in immobility time of mice in both FST and TST (Conc. 125 and 250 mg/kg) and antidepressant-like activities were noted. There was also an increase in the monoamines level.

2.25.37. Anti-epileptic Activity of *Smilax china*

Madhavan et al., (2008) assessed the anti-epileptic potential of roots and rhizome of *S. zeylanica* using alcohol and aqueous extracts by induced convulsion through PTZ (Pentylenetetrazole) and MES (Maximal electroshock) Method models in Swiss albino mice. Both the extracts delayed the onset of convulsions in PTZ induced seizures at dose of 600 mg/kg, whereas in maximal electroshock induced seizures, there was reduction in duration of extensor phase and time taken for recovery.

2.25.38. Pancreatic Lipase Activity of *Smilax china*

Cyclohexane, ethyl acetate and methanol extract of *S. zeylanica* was analyzed for pancreatic lipase activity by using porcine pancreatic lipase inhibition assay. The extracts showed a dose dependent action among the three-methanol extract showed highest % inhibition by 77.07% which was closer to the % inhibition of the standard orlistat i.e., 82.39% at 200 µg/ml whereas the % inhibition of cyclohexane and ethyl acetate was 37.52 and 54.44% respectively (Mishra et al., 2021).

2.25.39. Toxicity study of *Smilax china*

In acute toxicity study, ethanolic extract (EESC) and ethyl acetate fraction (EAF) of S. chinensis did not cause any mortality at a dose of 2 g/kg in mice. There were no gross

changes in behavior at higher doses. Hence, the plant extract was reported to have least or no toxic effects even at high doses (Zainab et al., 2019).

3.0. MATERIALS AND METHODS

3.1. Collection and identification

Smilax china was to collected from Moulovibazar, Dhaka. The plant was identified and authenticate by pharmacognosy department of Hamdard University Bangladesh.

3.2. Reagents and chemicals

Reagents and chemicals such as Wagner's reagent (Iodine in Potassium Iodide): Hagger's reagent (saturated picric acid solution): Mayer's reagent (Potassium Mercuric Iodide): Draggendrof's reagent (Solution of Potassium Bismuth Iodide): Molisch's reagent (Alcoholic α-naphthol solution): Benedict's Reagent, Fehling's reagent, Salkowski reagent, Concentrated sulphuric acid, 10% lead acetate, 0.1% ferric chloride, Fehling's solution, Dilute NaOH, Ammonia, Dilute HCl, Distilled water & Methanol was used in this study.

3.3. Preparation of plant material

Firstly, the plant material was washed with tap water for 3-4 times to remove any traces of soil particles and other dirt and then with distilled water for two times. After washing, plants will be kept in the shade for drying at room temperature and under the constant observation to avoid any contamination. Dried materials will be crushed with the help of electric grinder and stored in dry and cool place with proper labeling for further use.

3.4. Preparation of Extraction

The grinded 200 gm powder taken into clean glass containers and was soaked in 500 ml 100% Methanol. Then the container was sealed and kept for 3 days with occasional shaking and stirring. The whole mixture was filtered by a piece of clean, white cotton material and then the filtrate was further filtered with Whitman filter paper No.1 filter paper and then dry under shade (Sawant et al., 2013). It was used for the qualitative analysis of secondary metabolites.

3.5. Phytochemical Analysis

The crude extract was subjected to different chemical tests to find out the qualitative presence of phytochemical which was identified by characterized color changes using standard procedure as described for alkaloids (Harborne, 1973): steroids (Trease & Evans, 1989):

phenolics and flavonoids (Awe & Sodipo, 2001): saponins and cardiac glycosides (Sofowora, 1993): tannins (odebiyi & Sofowora, 1978): terpenoids and saponins (Obadonia & Ochuko, 2001).

3.5.1. Detection of alkaloids: Extracts was dissolved in dilute Hydrochloric acid and be filtered with Whitman filter paper No.1 filter paper.

a) Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (Solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager's Test: Filtrates was treated with Hager's reagent (Saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

3.5.2. Detection of carbohydrates: Extracts was dissolved in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

b) Benedict's Test: Filtrates was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. For action of red precipitate indicates the presence of reducing sugars.

3.5.3. Detection of glycosides: Extracts was hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test: Extracts was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cool and extracted with equal volumes of benzene. The benzene layer was separate and treat with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

b) Legal's Test: Extracts was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

3.5.4. Detection of saponins

a) Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

a) Foam Test: Extracts was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

3.5.5. Detection of phytosterols

a) Salkowski's Test: Extracts was treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

b) Libermann Burchard's test: Extracts was treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

3.5.6. Detection of phenols

a) Ferric Chloride Test: Extracts was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

3.5.7. Detection of tannins

a) Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

3.5.8. Detection of flavonoids

a) Alkaline Reagent Test: Extracts was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

3.5.9. Detection of proteins and aminoacids

a) Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

b) Ninhydrin Test: To the extract, 0.25% ninhydrin reagent ($C_9H_6O_4$) was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

4.0. RESULTS AND DISCUSTION

4.1. Phytochemical Screening of *Smilax china.*

Table 4.1. Results of alkaloids in methanolic extract of *Smilax china.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Alkaloids	Mayer's Test	Positive
2		Wagner's Test	Positive
3		Dragendroff's Test	Positive
4		Hager's Test	Positive

Figure-4.1. Mayer's Test

Figure-4.2. Wagner's Test

Figure-4.3. Dragendroff's Test

Figure-4.4. Hager's Test

Table 4.2: Results of carbohydrates in methanolic extract of *Smilax china.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Carbohydrates	Molisch Test	Positive
2		Benedict's Test	Positive
3		Fehling Test	Positive

Figure-4.5. Molisch's Test

Figure-4.6. Benedict's Test

Figure-4.7. Fehling's Test

Table 4.3: Results of glycoside in methanolic extract of *Smilax china.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Glycoside	Modified Borntrager's Test	Positive
2		Legal's Test	Positive

Figure-4.8.
Modified Borntrager's Test

Figure-4.9.
Legal's Test

Figure-4.10.
Froth Test

Figure-4.11.
Foam Test

Table 4.4: Results of saponins in methanolic extract of *Smilax china*.

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Saponins	Forth Test	Positive
2		Foam Test	Positive

Table 4.5: Results of phytosterol in methanolic extract of *Smilax china*.

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Phytosterol	Salkowski's Test	Positive
2		Libermann Burchard's Test	Positive

Table 4.6: Results of phenols in methanolic extract of *Smilax china*.

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Phenol	Ferric Chloride Test	Positive

Table 4.7: Results of tannins in methanolic extract of *Smilax china*.

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Tannins	Gelatin test	Negative

Figure-4.12.
Salkowski's Test

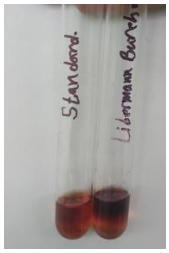


Figure-4.13.
Libermann Burchard's Test

Figure-4.14.
Ferric Chloride Test

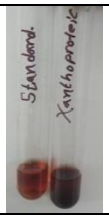
Figure-4.15.
Gelatin Test

Table 4.8: Results of flavonoids in methanolic extract of *Smilax china*.

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Flavonoids	Alkaline Reagent Test	Positive
2		Lead acetate Test	Positive

Table 4.9: Results of proteins and amino acids in methanolic extract of *Smilax china*.

Sl.	Detection of Phytochemicals	Name of the test	Result
1	**Proteins & Amino acids**	Xanthoproteic Test	Negative
2		Ninhydrin Test	Negative

			
Figure-4.16. Alkaline Reagent Test	**Figure-4.17. Lead acetate Test**	**Figure-4.18. Xanthoproteic Test**	**Figure-4.19. Ninhydrin Test**

4.2. DISCUSSTION

In my study, the qualitative phytochemical analysis of methanolic extract of *Smilax china* Table 4.1 showed the presence of alkaloids. One study conducted by Sabarisenthil et al., 2017, found the qualitative phytochemical analysis of methanolic extracts revealed the presence of alkaloids. Another study conducted by Apada et al., 2022, found the qualitative phytochemical analysis of ethanolic extracts revealed the presence of alkaloids. Both studies were coincided each other.

In my research, methanolic extract of *Smilax china* was subjected to a qualitative phytochemical examination. Carbohydrates were present, as shown in Table 4.2. In one investigation, carried out by Saravanakumar et al., 2014, it was discovered that the presence of carbohydrates was shown by the qualitative phytochemical analysis of methanolic extracts. Sabarisenthil et al's qualitative phytochemical analysis of methanolic extracts confirmed the presence of carbohydrates, according to another study they did in 2017. The two studies were related to one another.

A qualitative phytochemical analysis of a methanolic extract of *Smilax china* was performed in my study. Table 4.3 demonstrated the presence of glycosides. In one investigation, Sabarisenthil et al., 2017, found that qualitative phytochemical examination of methanolic extracts verified the presence of glycosides. Another study conducted by Bhati et al., 2011, Preliminary phytochemical tests of *Smilax china* in ethanol extract and water extract relevant the presence of glycoside. Both studies were almost similar.

The presence of saponin was revealed in my study's qualitative phytochemical analysis of the methanolic extract of *Smilax china* Table 4.4. in one study Sabarisenthil et al., 2017, discovered that the presence of saponin was indicated by the qualitative phytochemical analysis of methanolic extracts of *Smilax china.* Another study conducted by Bhati et al.,

2011, preliminary phytochemical tests of *Smilax china* in ethanol extract and water extract relevant the presence of saponins. Both researches were on the same wavelength.

As part of my research, the methanol extract of *Smilax china* was phytochemically tested. Phytosterols were found to be present in Table 4.5. In one study, Apada et al., 2022, found that phytosterol testing of ethanolic extracts confirmed the existence of phytosterols. Both studies were on the same track.

In my study, the qualitative phytochemical analysis of methanolic extract of *Smilax china* Table 4.6 showed the presence of phenol. One study conducted by Apada et al., 2022 confirmed the presence of phenol in ethanolic extract of *Smilax china*. The two studies were related to one another.

The absence of tanin was revealed in my study's qualitative phytochemical analysis of the methanolic extract of *Smilax china* Table 4.7. One study conducted by Sabarisenthil et al., 2017, found the qualitative phytochemical analysis of ethanolic extracts of *Smilax china* revealed the abesence of tannin. Another study conducted by Bhati et al., 2011, found the qualitative phytochemical analysis of petroleum ether extract, chloroform extract and water extract of *Smilax china* revealed the absence of tannin. Both studies were coincided each other.

In my study, phytochemical analysis of the methanol extract of *Smilax china* Table 4.8. revealed the presence of flavonoids. A study by Sabarisenthil et al., 2017, found that phytochemical analysis of the methanol extract of *Smilax china* revealed the presence of flavonoids. Another study conducted by Saravanakumar et al., 2014, found that phytochemical analysis of the methanol extract of *Smilax china* relevant the presence of flavonoids. The two studies coincide.

In my study, phytochemical analysis of the methanolic extract of *Smilax china* Table 4.9. revealed the absence of proteins & amino acids. A study by, apada et al., 2022, found that phytochemical analysis of the methanolic extract of *Smilax china* revealed the absence of proteins & amino acids. Both researches were heading in the same direction.

5. CONCLUSION

Smilax china, commonly known as China root or tufuling, is a medicinal plant widely used in traditional medicine. It belongs to the family Smilacaceae and is

characterized by its woody vine-like stems and heart-shaped leaves. The plant is primarily found in East Asia, including China, Japan, and Korea. *Smilax china* is a widely used medicinal plant in traditional medicine, known for its numerous health benefits. The plant contains various bioactive compounds that exhibit antioxidant, anti-inflammatory, and anti-tumor properties. The rhizomes are bitter, acrid, thermogenic, anodyne, anti-inflammatory, digestive, laxative, depurative, diuretic, febrifuge and tonic. It is used in dyspepsia, flatulence, colic, constipation, helminthiasis, skin diseases, leprosy and psoriasis, syphilis, strangury, seminal weakness, general debility, detoxifies organs, cleanses blood, aids absorption and kills bacteria; the plant has also been shown to possess potential anticancer effects against different types of cancer cells. Despite the promising findings, more research is needed to fully understand the Chemical composition, mechanisms of action and potential side effects of *Smilax china*. Phytochemical analysis of the extracts showed the presence of alkaloids, carbohydrates, glycoside, saponin, phytosterol, phenols, flavonoids and tannins, proteins & amino acid were absence. These findings align with the plant's historical use in traditional medicine, supporting its medicinal significance. Moreover, the presence of bioactive compounds with known biological activities suggests that *Smilax china* holds promise for various health applications. It is also used for fever, epilepsy, insanity, neuralgia and stimulates digestion, increases urination, protects liver and promotes perspiration. In Chinese medicinal science, it has been used for clinical treatment of syphilis, acute bacillary dysentery acute, chronic nephritis and antitumor. On the basis of scientific literatures, *S. china L*. demonstrates important and promising health benefits. In general, treatment with natural and traditional medicine, especially *S. china L* is recommended. However, further research is needed to quantify these phytochemicals, conduct pharmacological assays, and explore specific medicinal uses. Sustainable harvesting and conservation efforts are also crucial to ensure the continued availability of this valuable indigenous medicinal resource. The present investigation discovered that the *Smilax china* is a potential source of useful drugs due to the presence phytochemicals and can be utilizing in the treatment of many diseases/ailments and also be developing for use in the pharmaceutical industries.

6.0. Conflicts of interests

The authors do not disclose any conflicting interests.

7.0. ACKNOWLEDGMENT

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