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PHYTOCHEMICAL ANALYSIS OF BIOACTIVE COMPOUNDS OF *SYZYGIUM CUMINI* (L.) SKEELS.

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ABSTRACT

Syzygium cumini (L.) Skeels is one of the important medicinal plants widely used for the treatment of various diseases in particular diabetes. The present study was aimed to investigate the preliminary phytochemical screening of the leaves, stem bark, fruit pulp and seeds were demonstrated. The results revealed the presence of medicinally important phytochemical constituents in different organic solvents of hexane, chloroform, ethyl acetate and ethanol. The phytochemical tests of these extracts have showed the presence of alkaloids, flavonoids, phenols, tannins, terpenoids carbohydrate, tannin and saponin. The plant is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. The seeds are claimed to contain alkaloid, jambosine, and glycoside jambolin or antimellin, which halts the diastatic conversion of starch into sugar. Thin layer chromatographic profiles yielded the different pattern of compound and as well as different Rf values. The extracted bioactive compounds were tested followed by calculate their Rf value by analyzing thin layer chromatographic techniques with two different kinds of solvent systems.

KEYWORDS: Phytochemicals, *Syzygium cumini*, Leaf, Stem bark, Fruit pulp and Seed extracts.

INTRODUCTION

Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different part of country.[1] The medicinal use of plants is probably as old as mankind plants have continued to be a valuable source of natural products for maintaining human health, as studies natural therapies have intensified. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. More than 150,000 plants species have been studied and several of them contain therapeutic substances and the use plant compounds for pharmaceutical purposes has gradually increased. About 1500 plants are systematically used in indigenous system of medicine, like ayurveda, unani and siddha. It is ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing.[2] Herbalists use the leaves, flowers, stems, berries and root of plants to prevent, relieve and treat illness.[3] A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources.[4, 5]

According to World Health Organization, medicinal plants are probably the best source of variety of drugs. About 80% of individuals in developed countries use traditional medicine containing compounds derived from medicinal plants.[6] Various societies across the world have shown great interest in curing diseases using plants/plant based drugs. Over three-quarters of the world population relies mainly on plants and plant extracts for traditional knowledge systems related to the use of plant species and has an concept of using plants for medicinal purpose was been from very ancient period started before 2500 and 500 BC. This herbal and natural products have been used in folk medicine for centuries throughout the world, but there are relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, this coupled with their reduced cost, is encouraging for both the consuming public and national health care institutions to consider plant medicines as alternative to synthetic drugs.[7] The phytochemical analysis of the medicinal plants are important and have commercial interest in both research institutes and pharmaceutical companies for the manufacturing of the new drugs for treatment of various diseases. *Syzygium cumini* leaf, fruit pulp and bark and it justifies their use in the traditional medicines for the treatment of different diseases. There arises a need and therefore to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies.

With advances in photochemical techniques, several active principals of many medicinal plants have been isolated and introduced as valuable drug in modern systems of medicine.

Syzygium cumini (L.) Skeels belonging to family Myrtaceae. The plant parts such as seed, fruit, leaves, flower, bark used in folk medicine. The genus comprises about 1100 species, and has a native range that extends from Africa and Madagascar through southern Asia east through the Pacific. Its highest levels of diversity occur from Malaysia to northeastern Australia, where many species are very poorly known and many more have not been described taxonomically.[8, 9] Charaka used seeds, leaves and fruits in decoctions for diarrhoea and the bark as an astringent. Sushruta prescribed the fruit internally in obesity, in vaginal discharges and menstrual disorders, cold infusion in intrinsic haemorrhage. The juice of Jambu, Amra and Amalaka leaves mixed with goat milk and honey prescribed in diarrhoea with blood. [10] Leaf juice is taken orally to treat diabetes. The juice is taken mixed with milk every morning. Fresh leaf juice is taken orally for stomach pain.[11] The bark is astringent, its juice is given in chronic diarrhoea, dysentery, menorrhagia. According to Ayurveda, its bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic and in good for sore throat, bronchitis, asthma, thirst, biliousness, dysentery, blood impurities and to cure ulcers.[12] Decoction of the bark is an efficacious mouth-wash and gargle for treating spongy gums, stomatitis, relaxed throat and other diseases of mouth. The bark is used in dyeing and tanning and for colouring fishnets. The stem bark is rich in betulinic acid, friedelin, epi-friedelanol, β-sitosterol, eugenin and fatty acid ester of epi-friedelanol,[13] β-sitosterol, quercetin kaempferol, myricetin and gallic acid and ellagic acid,[14] bergenins,[15] flavonoids and tannins.[16] The presence of gallo- and ellagi-tannins may be responsible for the astringent property of stem bark.

Syzygium cumini is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. The seeds are claimed to contain alkaloid, jambosine, and glycoside jambolin or antimellin, which halts the diastatic conversion of starch into sugar and seed extract has lowered blood pressure by 34.6% and this action is attributed to the ellagic acid content.[17] The seeds have been reported to be rich in flavonoids, a well-known antioxidant, which accounts for the scavenging of free radicals and protective effect on antioxidant enzymes[18, 19] and also found to have high total phenolics with significant antioxidant activity and are fairly rich in protein and calcium. The fruit pulps are rich in sugar, mineral salts, vitamins C, which fortifies the beneficial effects of vitamin C,

anthocyanins and flavonoids.[20] Syrup prepared from the juice of the ripe fruit is a very pleasant drink. Syrup or vinegar prepared from the ripe fruit is useful in spleen enlargement and efficient astringent in chronic diarrhoea. Hot water extract of dried fruits is used for stomach ulcers, reduce acidity and for diabetes.[21]

The fruits are rich in raffinose, glucose, fructose,[22] citric acid, mallic acid,[23] gallic acid, anthocyanins;[24] delphinidin-3-gentiobioside, malvidin-3-laminaribioside, petunidin-3-gentiobioside, the color of the fruits might be due to the presence of anthocyanins,[25] cyanidin diglycoside, petunidin and malvidin.[26] The sourness of fruits may be due to presence of gallic acid. Hence, the present study has been made to investigate the preliminary phytochemical analysis of *Syzygium cumini* leaves, stem bar, fruit pulp and seeds extracted with different organic solvent systems.

MATERIAL AND METHODS

Collection of sample

The plant samples such as leaves, stem bark, fruit pulp and seeds of *Syzygium cumini* (L.) Skeels. were collected from Hogenakkal Hills of Dharmapuri district Tamilnadu, India during June to August 2015 and identified the voucher specimen has been deposited in departmental herbarium, PG and Research Department of Botany, Government Arts College, Dharmapuri, Tamil Nadu, India for future reference.

Preparation of extractions

Fresh leaves, stem bark, fruit pulp and seeds were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. They were ground into coarse powder by using mechanical pulveriser. All the samples, about 100g of the powder were repeatedly extracted with different solvents in a 500 ml round bottom flask with 250 ml solvents hexane, chloroform, ethyl acetate and ethanol separately. The reflux time for each solvent wash 25 cycles for complete extraction using soxhlet apparatus.[27] The extraction was done in soxhlet apparatus at 60°C for 6 hour. After the completion of extraction, the supernatant was filtered through Whatman No. 1 filter paper. All solvent extracted fractions were evaporated to dryness to obtain residues. The extracts were stored at 4°C in air tight containers and used for preliminary phytochemical screening of secondary metabolites. The presence of different chemical constituents in crude drugs can be detected by subjecting them to successive extraction using solvents in the order of increasing polarity. In the present study

were therefore, subjected to extraction followed by qualitative chemical tests in order to know the phyto profiles on a preliminary basis.

PHYTOCHEMICAL SCREENINGS[28, 29]

Phytochemical screening were performed to assess the qualitative chemical composition of different samples of crude extracts using commonly employed precipitation and coloration reactions to identify the major secondary metabolites like alkaloids, glycosides, steroids, tannins and terpenoides. To identify the chemical constituents of sample extracts by standard producers have been followed. The crude extract was qualitatively tested for the presence of chemical constituents using the following reagents and chemicals. The extracts obtained in the successive extraction process were subjected to various qualitative tests using reported methods, subjected to preliminary phytochemical screening for the identification of various phytoconstituents such as alkaloids, carbohydrates, steroids, cardiac glycosides, flavonoides, carbohydrates, amino acids, phenols, naphthoquinones and tannins according to standard methods.

Tests for Alkaloides

Dragendroff's test: Little amount of the sample was treated with the Dragendroff's reagent; the appearance of reddish brown precipitate indicated the presence of alkaloids.

Mayer's test: Sample (2-3ml) was treated with few drops of Mayer's reagent. Appearance of white precipitate indicated the presence of alkaloids.

Wagner's test: Sample (2-3ml) was mixed with few drops of Wagner's reagent. Appearance of reddish brown precipitate indicated the presence of alkaloids.

Tests for Carbohydrates[30]

Molisch's test: To a small amount of the extract few drops of Molish's reagent was added followed by the addition of conc. H_2SO_4 along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 ml of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates. First yellow then brick red precipitate was observed.

Fehling's test: The extract was treated with 5 ml of Fehling's solution (A and B) and kept in boiling water bath for 5-10 min. The formation of yellow or red colour precipitate indicates the presence of reducing sugar.

Benedict's test: Sample solution and equal volume of Benedict's reagent were mixed in the test tube. Heated in boiling water bath for 5 min solution appears green, yellow colour appeared based on the amount of reducing sugar present in test solution.

Test for Glycosides:[31] Free content of the sugar extract was determined. The sample was hydrolysed with mineral acid (dilute hydrochloric or dilute sulphuric acid). Again the total sugar content of the hydrolysed extract was determined. Increase in the sugar content indicated the presence of glycoside in the extract.

Glycoside -----H_2O----→ Aglycon (genin) + Glycon (sugar)

Baljet's Test: To 5 ml of the extract few drops of sodium picrate was added to observe yellow to orange colour.

Keller-killiani test: To 5 ml of the extract few drops of ferric chloride solution was added and mixed, then sulphuric acid containing ferric chloride solution was added, it forms two layer showed reddish brown while upper layer turns bluish green indicates the presence of glycosides.

Legal's test: Aqueous or alcoholic sample extract was mixed with 1 ml of pyridine sodium nitroprusside was added. Pink to red color appeared.

Test for steroids[31]

Salkowski's test: Sample (2 ml) was mixed with 2 ml of concentrated Sulphuric acid, it was well shaken then chloroform layer appeared red and acid layer shown greenish yellow fluorescene.

Liberman-Buchard reaction: Sample (2 ml) was mixed with chloroform. 1-2 ml of acetic anhydride was added and 2 drops concentration sulphuric acid was added from the sides of the tube. First red then blue and finally green colour appeared.

Test for Proteins[31]

Million's test: Test sample (3ml) was mixed with 5ml of Million's reagent. White precipitate is formed. On warming precipitate turn's brick red or the precipitate dissolves giving red colored solution.

Biuret test: Test sample (3 ml) was mixed with 4% NaOH and few drops of 1% $CuSO_4$ solution were added. Violet or pink color not appeared. To 3 ml of the extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple colour. On addition of alkali, it becomes dark violet.

Test for flavonoides

Alkaline test: To 3 ml of the extract few magnesium ribbons are dipped and concentrated Hydrochloric acid was added over them and observed for the formation of magenta (brick red) colour indicating the presence of flavonoides.

Shinoda test: Sample extract was treated with 5 ml of 95% ethanol; few drops of concentrated Hydrochloric acid and 0.5g of magnesium turnings were also added. Pink colour was observed. Addition of increasing amount of sodium hydroxide to the residue shown yellow coloration, which decolorizes after addition of acid.

Determination of Flavonoides as Quecetin Equivalent: Flavonoids contents in the extracts were determined by standard colorimetric method with minor modifications. To 1 ml leaf extract was added 0.3 ml 5% sodium nitrite; 4 ml distilled water and held for 5 minutes. To the mixture 0.3 ml of 10% aluminium chloride was and held for 6 minutes. Finally 2 ml of 1M sodium.

Test for Tannins[31]

A fraction of the extract was dissolved in water and then it was subjected to water bath 37°C for 1 h and treated with ferric chloride solution and observed for the formation of dark green colour.

Lead acetate test: The sample was treated with 10% lead acetate solution; appearance of white precipitate indicated the presence of tannins. When the extract was treated with aqueous bromine solution, appearance of white precipitate indicated the presence of tannins.

Ferric chloride test: To 1 ml of extract, 2 ml of 5% ferric chloride was added. Formation of greenish black colour indicated the presence of tannins. A fraction of the extract was dissolved in water and then it was subjected to water bath 37°C for 1hr and treated with ferric chloride solution and observed and for the formation of dark green colour.

Test for Saponin[31]

Foam test: To 1 ml of the extracts 5 ml distilled water was added and shaken vigorously. Formation of foam indicated presence of saponins.

Test for anthraquinones: Weighted leaf powder, 0.5g, was boiled in 10% hydrochloride acid and filtered hot. To this, 2 ml chloroform and 10% ammonia solution each were added. Formation of pink color in the aqueous layer indicated presence of anthraquinones.

Test for Amino Acids[31]

Ninhydrin test: Test sample (3ml) and 3 drops of 5% ninhydrin solution were heated in boiling water for 10 min. Purple color appeared.

Test for phenols[31]

Ferric chloride test: A fraction of the extract was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour.

To 1 ml of the extract, 2 ml of distilled water, 3 drops of 10% aqueous ferric chloride ($FeCl_3$) and 3 drops of potassium ferrocyanide were added. Formation of blue or green color showed the presence of polyphenols.

Test for Total Polyphenols: Phenolic compounds in the leaf extracts were estimated by a colorimetric assay, based on standard procedures described by Harbone (1998) with minor modifications. To 5 ml distilled water was added 0.5 ml Folin Ciocalteu's reagent. After 3 min, 1 ml 7.5% sodium carbonate solution, 1 ml extract were added to the mixture and made to 10 ml with distilled water. The mixture was kept in water bath maintained at 50°c for 16 minutes. UV Visible spectrophotometer (UV-Vis Shimadzu) was used to read the absorbance at 765 nm. Gallic acid was prepared in different concentrations and the absorbance equally read at 765 nm. The values obtained were used to generate the standard curve against which polyphenols in the leaves, stem bark, fruit pulp and seeds were calculated and expressed as Gallic acid equivalents (GAEs) per 100 g dwb.

Test for terpenoides[31]

Chloroform test: To 5 ml of the extract few drops of chloroform and concentrated H_2SO_4 was added carefully along the sides of the test tube. Formation of brown color at interface was a positive indicator.

Test for reducing sugars: The residue was dissolved in water and kept in the water bath. Two ml of the solution in a test tube was added with 1 ml each of Fehling's reagent A and B. The mixture was shaken and heated in a water bath for 10 min. A brick red precipitate indicates a reducing sugar.

IDENTIFICATION OF COMPOUND BY THIN LAYER CHROMATOGRAPHY (TLC)

In 1958 Stahl[32] demonstrated application of TLC in analysis. It is at present an important analytical tool for qualitative analysis of a number of natural products. The plates were visualized for spot identification under iodine chamber and sprayed with spray reagent of the category given in table. The Rf value was calculated by using formula.

$$\text{Rf value} = \frac{\text{Distance travelled by solute from the base line}}{\text{Distance travelled by solvent from the base line}}$$

10 mg/ml of *Syzygium cumini* in different extract was dissolved in ethanol solvent used for TLC examination. TLC plates were prepared by using Silica Gel-G as adsorbent. 100g Silica Gel-G was mixed with sufficient quantity of distilled water to makes Slurry. The slurry was immediately poured into a spreads and plates were prepared by spreading the slurry on glass plates of required size. Plates were allowed to air dry for one hour.

RESULTS

The present study was carried out to analyze the presence of bioactive compounds in leaves, stem bark, fruit pulp and seeds of *Syzygium cumini*. The presence of phytochemical compounds in the plant extracts of *S. cumini* were evaluated by hexane, chloroform, ethyl acetate and ethanol extracts. This investigation through cold percolation and Soxhlet extraction method revealed the presence of saponins, tannins flavonoids, reducing sugars and steroids. The concentrated all extracts of *S. cumini* were carefully stored and analyzed. The colours of the extracts were pale green colour to yellow and brown in colour particularly chloroform extract was bright yellow in colour. The percentage yield of these extract were also measured, and it was the ethanolic extract 26.47% maximum yield in comparison with other solvent extracts. All the extracts were stickey semisolid in their consistency.

The preliminary phytochemical screening of the hexane, chloroform, ethyl acetate and ethanol extracts were revealed the presence of alkaloids, amino acids, glycosides, phytosterols, triterpenoids, steroids, saponins, flavonoids, phenol, tannins in different

concentrations except carbohydrates from different solvent extracts. Among the four solvents used for extraction, ethanol extracts were yielded maximum bioactive compounds followed by ethyl acetate extract, chloroform extract and minimum level of compounds present in hexane extract respectively. Plant parts concern the stem bark and seeds were showed more phytochemicals than leaf and fruit pulp extracts. The plant bark revealed the presence of flavonoids, tannins, sugars, proteins, amino acids, sterols and absence of steroids, alkaloids, saponins, terpenoids (Table 1). Further, the ethyl acetate and ethanol extracts were showed the absence of anthraquinones. Terpenoids and phytostrols were present in stem bark, seed and fruit pulp extract but absent in leaf extract. Anthraquinon glycosides were present in leaf and bark extracts but absent in seed and pulp extracts. Proteins were the highest constituents in all types of solvent. However, carbohydrates were absent in all extracts of the leaves.

For quantification of phytochemicals and its Rf values were calculated by thin layer chromatographic analysis. Further individual bands and its colour and Rf value based on corresponding authentic samples of each bioactive compounds have been identified. Thin layer chromatographic profiles yielded the different pattern of compound and as well as different Rf values. The extracted bioactive compounds were tested followed by calculate their Rf value by analyzing thin layer chromatographic techniques with two different kinds of solvent systems. The number of bands and Rf values of each extracts and in suitable solvent systems were presented in Table 2. Two types of solvent systems were used to evaluate better elution of compounds. The ethanol extracts of leaves and stem bark have yielded four compounds in leaves (Rf = 0.16, 0.35, 0.48, 0.65) and stem bark (Rf = 0.18, 0.36, 0.47, 0.63) respectively followed by three compounds in ethyl acetate extract (0.30, 0.62, 0.72) and (0.28, 0.60, 0.67), two compounds in chloroform (0.44, 0.54) and (0.38, 0.49), and only one compound yielded in hexane extract (0.91 and 0.90) in Benzene : Ethanol : Acetone (8:1:1) solvent system respectively. Similarly the other solvent system Benzene : Ethanol : Acetone (7:2:1), the ethanol extract showed four compounds in leaves (Rf = 0.13, 0.33, 0.45, 0.63) and in stem bark (Rf = 0.17, 0.35, 0.49, 0.62) respectively followed by three compounds in ethyl acetate extract (0.27, 0.54, 0.72) and (0.25, 0.55, 0.69), two compounds in chloroform (0.40, 0.51) and (0.40, 0.48), and only one compound yielded in hexane extract (0.86 and 0.82).

The ethanol extracts of fruit pulp and seeds have yielded four compounds in fruit pulp (Rf = 0.25, 0.42, 0.62) and seeds (Rf = 0.24, 0.45, 0.68) respectively followed by three compounds

in ethyl acetate extract (0.29, 0.61, 0.70) and (0.31, 0.58, 0.65), two compounds in chloroform (0.38, 0.47) and (0.40, 0.52), and only one compound yielded in hexane extract (0.88 and 0.87) in Benzene : Ethanol : Acetone (8:1:1) solvent system respectively. Similarly the other solvent system Benzene : Ethanol : Acetone (7:2:1), the ethanol extract showed four compounds in fruit pulps (Rf = 0.26, 0.51, 0.72) and in seeds (Rf = 0.28, 0.55, 0.74) respectively followed by three compounds in ethyl acetate extract (0.28, 0.58, 0.75) and (0.30, 0.55, 0.73), two compounds in chloroform (0.36, 0.47) and (0.35, 0.51), and only one compound yielded in hexane extract (0.85 and 0.84) (Table 3).

Table 1: Preliminary phytochemical analysis of Leaf, Stem bark, Fruit pulp and Seeds extracts of *Syzygium cumini*.

Biochemical tests	Leaf				Stem bark				Fruit pulp				Seed			
	H	C	A	E	H	C	A	E	H	C	A	E	H	C	A	E
Alkaloids: Mayer's Test	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+
Wagner's Test	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+	+
Dragendroff's Test	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+	+
Carbohydrates: Molisch's Test	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+
Fehling's test	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
Benedict's Test	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
Glycosides: Baljet's Test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Keller-Killiani test	+	+	+	+	-	-	+	+	-	-	+	+	-	-	-	-
Legal's test	+	+	+	+	-	-	+	+	-	-	+	+	-	-	-	-
Steroids: Liebermann-Buchard Test	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	+
Salkowskis Test	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
Proteins: Biruet test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Millions test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoides: Alkaline Test	+	+	+	-	+	+	-	-	+	+	-	-	+	+	-	-
Shinoda Test	+	+	+	-	+	+	-	-	+	+	-	-	+	+	-	-
Tannins: Lead acetate Test	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
Ferric chloride Test	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-
Saponins: Foam Test	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+
Anthraquinones: NH_4OH Test	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Amino acid:	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-

Ninhydrin test																
Phenols: Ferric chloride	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
Terpenoids: Chloroform Test	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+
Reducing sugars: Fehling's Test	-	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+

(Note: H=Hexane; C=Chloroform; A=Ethyl Acetate; E=Ethanol; (+) = Present; (-) = Absent)

Table 2. Thin Layer Chromatographic analysis of *Syzygium cumini* leaves and stem bark extracts with their Rf values in different solvent systems.

S. No.	Solvent system	Leaf extract	No. of bands	Rf values	Stem bark extracts	No. of bands	Rf values
1.	Benzene : Ethanol : Acetone (8:1:1)	Hexane	1	0.91	Hexane	1	0.90
		Chloroform	2	0.44	Chloroform	2	0.38
				0.54			0.49
		Ethyl acetate	3	0.30	Ethyl acetate	3	0.28
				0.62			0.60
				0.72			0.67
		Ethanol	4	0.16	Ethanol	4	0.18
				0.35			0.36
				0.48			0.47
				0.65			0.63
2.	Benzene : Ethanol : Acetone (7:2:1)	Hexane	1	0.86	Hexane	1	0.82
		Chloroform	2	0.40	Chloroform	2	0.40
				0.51			0.48
		Ethyl acetate	3	0.27	Ethyl acetate	3	0.25
				0.54			0.55
				0.72			0.69
		Ethanol	4	0.13	Ethanol	4	0.17
				0.33			0.35
				0.45			0.49
				0.63			0.62

Table 3: Thin Layer Chromatographic analysis of *Syzygium cumini* fruit pulp and Seeds extracts with their Rf values in different solvent systems.

S. No.	Solvent system	Fruit pulp extracts	No. of bands	Rf values	Seed extracts	No. of bands	Rf values
1.	Benzene : Ethanol : Acetone (8:1:1)	Hexane	1	0.88	Hexane	1	0.87
		Chloroform	2	0.38	Chloroform	2	0.40
				0.47			0.52
		Ethyl acetate	3	0.29	Ethyl acetate	3	0.31
				0.61			0.58
				0.70			0.65
		Ethanol	3	0.25	Ethanol	4	0.24
				0.42			0.45

				0.62			0.68
2.	Benzene : Ethanol : Acetone (7:2:1)	Hexane	1	0.85	Hexane	1	0.84
		Chloroform	2	0.36	Chloroform	2	0.35
				0.47			0.51
		Ethyl acetate	3	0.28	Ethyl acetate	3	0.30
				0.58			0.55
				0.75			0.73
		Ethanol	3	0.26	Ethanol	3	0.28
				0.51			0.55
				0.72			0.74

DISCUSSION

In the present study, most of the biologically active phytochemicals such as flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoids, cardiac glycosides and tannins were found to be present in the ethanolic extracts of different parts of the *S. cumini* plant. The medicinal properties of *S. cumini* plant extracts may be due to the presence of above mentioned phytochemicals. Studies on the efficiency of medicinal plants with respect to the control of infectious diseases are more essential to know their therapeutic value and hence in pharmaceutical arenas. Plants having antimicrobial compounds have enormous therapeutic potential as they can act without any side effect as often found with synthetic antimicrobial products.

The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance, flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities.[33-35] Plant steroids are known to be important for their cardiotonic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics. Tannins were reported to exhibit antiviral, antibacterial and anti-tumour activities. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic.[36, 37] Saponin is used as mild detergents and in intracellular histochemistal staining. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in hypercholestrolaemia, hyperglycaemia, antioxidant, anticancer, antiinflammatory, weight loss, etc. It is also known to have antifungal properties.[38, 39]

For instance, the flavonoids present in the stem bark of *Syzygium cumini* may have the free radical scavenging capacity, antioxidative activity, coronary heart disease prevention, anti-

inflammatory, antiviral, anticancer and hepatoprotective activities.[40] The tannins present in the stem bark of *Syzygium cumini* may responsible for anticarcinogenic, antimutagenic, antioxidative, antimicrobial activities, as well as reduce blood pressure, accelerate blood clotting, decrease the serum lipid level, modulate immune responses. The tannins also have been reported to be responsible for decreased intake of food, net metabolizable energy, growth rate, and protein digestibility in experimental animals this may be responsible for the decreased body weight noticed in the present study but not statistically significant compared to control group.[41] The presence of sterols has a pharmacological activity like lowering blood cholesterol and anti-inflammatory.[42]

The fruit contains 85.80 g moisture, 0.13 g protein, 0.30 g fat, 0.90 g crude fiber, 14.00 g carbohydrate, 0.40 g ash, 15.00 mg calcium, 35.00 mg magnesium, 16.20 mg phosphorus, 1.62 mg iron, 26.20 mg sodium, 55.00 mg potassium, 0.23 mg copper, 13.00 mg sulfur, 8.00 mg chlorine, 80 I.U. vitamin A, 0.03 mg thiamine, 0.01 mg riboflavin, 0.29 mg niacin, 18.00 mg ascorbic acid, 7.00 mg choline and 3.00 mcg folic acid per 100 g of edible portion (fruit pulp).[43] The peel powder of jambolan also can be employed as a colorant for foods and pharmaceuticals and anthocyanin pigments from fruit peels were studied for their antioxidant efficacy stability as extract and in formulations.[44]

The results obtained in our ethanol extraction method revealed the presence of flavonoids in all of the screened medicinal plant extracts and the presence of flavonoids could be extremely helpful as flavonoids possess antiallergic, antiinflamatory, antiviral and antioxidant activities.[45, 46] Moreover, acting by several different mechanisms, particular flavonoid can extract significant anticancer activity, amongst other modes of action. Certain flavonoids possess potent inhibitory activity against a wide array of enzymes. Evidence suggests that only activated cells are responding to a stimulus. So the presence of this type of phytochemical compounds in the screened medicinal plants has a wide range of applications and could be certainly used for a variety of applications.[47]

Phytochemical analysis conducted on the plant extracts has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Analysis of the plant extracts revealed the presence of phytochemicals such as alkaloids, glycosides, terpenoids, steroids, saponins, flavonoids and tannins except carbohydrates, results were summarized in Table 1. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. Several workers have reported the analgesic[48]

antispasmodic and antibacterial properties of alkaloids[49, 50] Glycosides are known to lower the blood pressure according to many reports.[51] The terpinoids have been shown to decrease blood sugar level in animal studies.[52]

Plant steroids are known to be important for their cardiotonic activities; they possess insecticidal and anti-microbial properties. They are routinely used in medicine because of their profound biological activites. Glycosides are nonvolatile and lack fragrance and serve as defense mechanisms against predation by many microorganisms, insects and herbivores. Plant saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccines and knock out some kinds of tumor cells, particularly lung and blood cancers. These compounds served as natural antibiotics, which help the body to fight infections and microbial invasion.[53]

The extracts of higher plants can be very good source of antibiotics[54] against various bacterial pathogens. Plant having antibacterial compounds have enormous therapeutic potential as they can act without any side effect as often found with synthetic antibacterial products. The results obtained in this study suggests a potential application of *S. cumini* leaves for treatment of skin wounds, typhoid and further investigations should be conducted in order to explore their applications. Other medicinal plants containing Phenolic compounds, including tannins, as major constituents are used topically for care and repair of skin wounds. The advantage of the use of topical antibacterial is their ability to deliver high local concentrations of antibiotic irrespective of vascular supply. Further benefits include the absence of adverse systemic effects, and a low incidence of resistance.[55]

CONCLUSION

The results of the present study revealed the presence of several phytochemical compounds like saponins, tannins and steroids. In the ethanolic extract shows the presence of maximum number of bioactive compounds yield such as alkaloids, carbohydrates, proteins, amino acids, Phenolic compounds and tannins. Similary alkaloids, carbohydrates, proteins, amino acids, Phenols were present in all four extracts. These phytochemical compounds may contribute as useful source of herbal and ayurvedic pathway for effective treatment of various diseases considering its tremendous potential pharmacological activites. From the above information it is clear that *S. cumini* possesses potential antidiabetic compounds which are of utmost importance and is therefore imperative to further investigation. Further studies should be

carried out in order to explore the exact mechanism of these compounds to use *Syzygium cumini* (L.) Skeels. as a potential antidiabetic medicinal plant.

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