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PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF SENNA ALATA LEAVES EXTRACT

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ABSTRACT

Objectives: Ethanolic extrat of *Senna alata leaves* leaves was investigated for antimicrobial activities. **Methods**: The well diffusion method was followed for antibacterial assay. Antimicrobial activity of the leaves extract (200µg,400µg, 600µg and 800 µg) using well diffusion method. In this disc was prepared by using whatmann No.1 filter paper. Then, the filter paper disc of 5mm diameter were sterilized and soaked in the different concentration of plant extract. **Results**: The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs and has necessitated the search for new antimicrobials from alternative Sources. In the present investigation, preliminary phytochemical analysis was carried out in the extracts of *Senna alata*. **Conclusion**: In the present investigation, preliminary phytochemical analysis was carried out in the extracts of *Senna alata*. The extracts showed the presence of alkaloids, reducing sugar, coumarin, tannin and phenolic compounds.

KEYWORDS: *Streptococcus pneumonia*, *Proteus.sp* and *Enterobacter sp.*

INTRODUCTION

In India over one and half million medical practitioners use medicinal plants in preventive, promotive and curative applications. In recent years, secondary plant metabolites (phytochemicals) with unknown pharmacological activities have been extensively investigated as a source of medicinal agents.[1] In the last one decade there has been a growing interest to search for phytochemicals of native and wild plants for pharmaceuticals and nutritional requirements. The plant extracts are used as alternative remedies for many infectious diseases. The ingredients found in plants are of interest because they possess antibiotic resistances. Many herbs have been reported to exhibit anti oxidant activities due to the presence of isoflavones, flavonoides and anthocyanin.

Herbal medicine involves the use of plants for medicin-al purposes. The term "Herb" includes leaves, stems, flowers, fruits, seeds, roots, rhizomes and bark. There can be little doubt that the use of plants for healing purposes is the most ancient form of medicine known. The quest for plants with medicinal properties contin-ues to receive attention as scientists are in need of plants, particularly of ethno botanical significance for a complete range of biological activities, which ranges from antibiotic to anticancerous. Several plants and herb species used traditionally have poten-tial antimicrobial and antiviral properties and this has raised the optimism of scien-tists about the future of phyto-antimicrobial agent.[2]

Infectious diseases are the world's leading human and animal killers. The situation is further complicated by the rapid development of multi-drug resistance to available antimicrobial agents.[3] In recent years, pharmaceutical companies have focused on developing drugs from natural products. Plants still remain the most effective and cheapest alternative sources of drugs.[4] Drug discovery must be a continuing process if effective chemotherapeutic agents against the rapidly increasing drug resistant bacteria and fungi are to be obtained. The local use of natural plants as primary remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa.[5] Demands of traditional herbal medicines are increasing day by day not only by the developing countries but also by the developed countries throughout the world. The use of herbal medicine predates the introduction of antibiotics and predates social, economic and religious barriers.[6] So studies on the ethno-botany, ethnophytopathology and ethnomedicinal uses of our wild medicinal plants and investigations regarding enhanced productivity of medicinal plants is one of the frontier areas

of modern research. Ethnobotany is the study of the relationship between people and plants.[7] This interdisciplinary field includes studying plants as wild foods and as agricultural crops. Many species of Sennia posses anti-tumours, laxative, emetic, astringent, anti-pyretic and anti-oxidant properties.[7] Senna alata -commonly known as ”dadmari/candlebrush” having very high medicinal values like, antimicrobial property particularly against fungal dermatophytes and traditionally being used in the treatment of skin infections in man.[8] Leaf extract is also credited for the treatment of constipation, inguinal hernia, intestinal parasitosis, syphilis and diabetes.[9]

Leaf extract is a good antioxidant and the compound obtained from has been identified as a flavonol compound and named as ‘Kaempferol’.[10] Senna alata is categorized under the family Fabaceae, a pan tropical ornamental shrub, 2-3m high, widely distributed in tropical countries, stretching from Tropical America to India, Fiji, Indonesia, Malaysia and Africa. Senna alata Linn (Fabaceae) is an ornamental shrub, which grows well in forest areas of West Africa. It is locally used in Nigeria in the treatment of several infections which include ringworm, parasitic skin diseases.[11] Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine.[12] Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential.[13] The use of medicinal plants with antimicrobial activities needs to be given more attention to arrest the situation. The demonstration of the antimicrobial activity of *Senna alata* in this work provides scientific basis for its use as a local health remedy. The plant can therefore be used in the treatment of gastrointestinal, urinary tract and wound infections as well as some mycotic infections. The plant is laxative, antibacterial, antitumor, anti-inflammatory, diuretic, analgesic, vulnerary, weakly antifungal, hypoglycaemic and antispasmodic. They are taken internally as a remedy for constipation and to purify the blood. They can be applied as a tincture; as a poultice; powdered, then mixed with oil as an ointment; or the sap can be spread over the affected area - they form an effective treatment for skin blemishes, scabies, ringworm and other fungal skin infections. The bark is used to treat skin diseases, diarrhoea, worms, parasitic skin diseases, scabies and eczema. The root is laxative. An infusion is used in the treatment of diarrhoea, tympanites, uterus problems and filaria worm expulsion. The root is applied externally to treat sores and skin fungi. The flowers are used as a laxative and vermifuge. An infusion is used for remedying spleen conditions. A decoction combined with *Zingiber officinale*, is used as a treatment for grippe and as an abortifacient. The seed is laxative and anthelmintic. It is cooked and used as a

remedy for intestinal worms. The leaf contains the purgative anthraquinone, and also shows some antimicrobial activity.

The present study was aimed to evaluated the antibacterial potential of methanol extract of *Senna alata leaves* against microbial pathogens and phytochemical analysis was done.

MATERIALS AND METHOD

COLLECTION OF PLANT MATERIAL

The healthy plant samples of *Senna alata leaves Linn* was collected from Trichy. The collected plant materials were transported to the laboratory.

PREPARATION OF LEAF POWDER

The *Senna alata leaves* was collected,washed and cut into small pieces and dried at room temperature for two weeks and made in to powder for further analysis.

EXTRACTION OF PLANT MATERIAL

Aqueous and alcoholic extracts were prepared according to the methodology of Indian pharmacopoeia. The shady dried plants materials were subjected to pulverization to get coarse powder. The coarse powder material was subjected to soxhlet extraction separately and successively with alcohol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°c). The aqueous and alcohol extracts put in air tight containers stored in a refrigerator.

ANTIMICROBIAL ACTIVITY

Micro organisms and culture media

The bacterial cultures such as, pseudomonas sp were obtained from doctor diagnostic center, Trichy. The bacterial strains were maintained on nutrient agar mediam.The antibacterial activity studied by agar well diffusion method.

ANTIBACTERIAL ASSAY

Sterile Petri plates containing 20 ml of Nutrient agar or Muller Hinton agar were seeded with 0.01ml of 18 hours old test bacterial culture with calibrated loop (Hi-media) and lawned evenly using sterile cotton swabs. Appropriate quantity of different extracts were dissolved in Dimethyl sulfoxide (DMSO) and sterilized by using syringe filter. 200µg, 400µg, 600µg, and 800µg/ well concentration of plant samples, positive controls and negative controls were added into the 6mm diameter well. Incubation was made at 37^{o} C for 24 hours. The

assessment of antibacterial activity was based on the measurement of diameter of the inhibition zone formed around the well, using Himedia scale. Streptomycin sulphates 100µg were used as a positive control. DMSO was used as a negative control.

RESULT AND DISCUSSION

Table 1: Preliminary Phytochemical Screening Of *Senna Alata*

S.NO	TEST	AQUEOUS	ETHANOL
1	Alkaloids	+	+
2	Anthaquinone	-	-
3	Coumarin	+	+
4	Flavonoids	-	+
5	Glycosides	-	+
6	Phenols	+	+
7	Saponin	+	+
8	Steroids	-	+
9	Tannins	+	+
10	Terpenoids	-	-

Table 2: Antibacterial Activity Of Ethanol Extract Of *Senna Alata Leaves* Against Pathogens.

S. No.	Oraganisms	Concentration of extract in µl/ zone of inhibition in mm					
		200 µg	400 µg	600 µg	800 µg	Positive control	Negative control
1	*Proteus.sp*	9.0	11.3	13.7	15.0	20.0	-
2	*Enterobactersp*	10.0	11.3	11.6	17.0	24.0	-
3	*S.pneumoniae*	11.6	11.8	12. 7	19.5	26.0	-

Table 3: Antibacterial Activity Of Aqueous Extract Of *Senna Alata Leaves* Against Pathogens.

S. No.	Oraganisms	Concentration of extract in µl/ zone of inhibition in mm					
		200 µg	400 µg	600 µg	800 µg	Positive control	Negative control
1	*Proteus.sp*	10.0	11.5	11.9	12.3	21.3	-
2	*Enterobactersp*	12.0	13.8	14.5	**14.8**	23.6	-
3	*S.pneumoniae*	10.7	11.8	12. 8	13.2	25.5	-

Table 4: Antibacterial Activity Of Silver Nano Particles (Agnps) Of *Senna Alata Leaves* Against Pathogens.

S. No.	Oraganisms	Concentration of extract in µl/ zone of inhibition in mm					
		200 µg	400 µg	600 µg	800 µg	Positive control	Negative control
1	*Proteus.sp*	11.7	12.3	12.7	**15.6**	21.0	-
2	*Enterobacter sp*	13.5	13.8	14.4	**18.0**	23.5	-
3	*S.pneumoniae*	11.0	12.1	15.2	**21.9**	26.0	-

The preliminary phytochemical analysis was carried out for the aqueous and alcohol extract of *Senna alata* leaves. The phytochemical analysis was carried out in the two different extract. The qualitative analysis of the ethanolic and water extracts of *Senna alata* revealed the presence of alkaloid, flavanoid, The water extract of *Senna alata* contains tannin and phenolic compound. Moreover, the highest yield was also observed in alcohol extract which includes alkaloids, flavonoids, coumarins, saponin, phenol, tannin, glycosides, and steroids, hence this was selected for further studies (Table1).

Our findings are supported by the following researches. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. The development of microbial resistance to antibiotics has led the researches to investigate the alternative sources for the treatment of resistant strains.

Saponin is used as a mild detergent and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hyperchloles-trolaemia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory, and weight loss etc. It is also known to have antimicrobial properties**.** Plant steroids are known to be important for their cardio tonic, insecticidal and anti-microbial properties. They are also used innutrition, herbal medicine; cosmetics and they are routinely used in medicine because of their profound biological activities**.** Tannin is reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also reported that certain tannin are able to inhibit HIV replication selectivity and is also used as diuretic**.** Plant tannin has been recognized for their pharmacological properties and is known to make trees and shrubs a difficult meal for many caterpillars.[14]

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Many of these indigenous medicinal plants are used as spices. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes.[15]

The flavonoids have long been recognized to possess antiallergic, anti-inflammatory, antibacterial, antiviral, anti-proliferative and anti-carcinogenic activities as well as to affect some aspects of mammalian metabolism. It gives protection against free radicals, platelet aggregation, microbes, ulcers and hepatoxins. The phytochemical analysis revealed the

presence of flavonoids in the herbal plants. Our plant also contains alkaloids, terpenoids and other phenolic compounds hence it might has exhibited antibacterial activity.[16]

Various herbs and spices have been reported to exhibit antioxidant activity and antimicrobial properties. A majority of the antioxidant antimicrobial properties is attributed to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins.

Phytochemicals obtained from vegetables, fruits, spices, teas, herbs and medicinal plants, such as alkaloids, terpenoids and other phenolic compounds, have been proven to suppress different bacterial pathogens incuding respiratory and urinary tract pathogens such as *Pseudomonas aeruginosa, Klebsiella pneumoniae,E.coli, Streptococcus pneumonia*.[17]

Plate: 1 shows the Phytochemical Analysis of water and alcohol extract of *Senna alata* leaves.

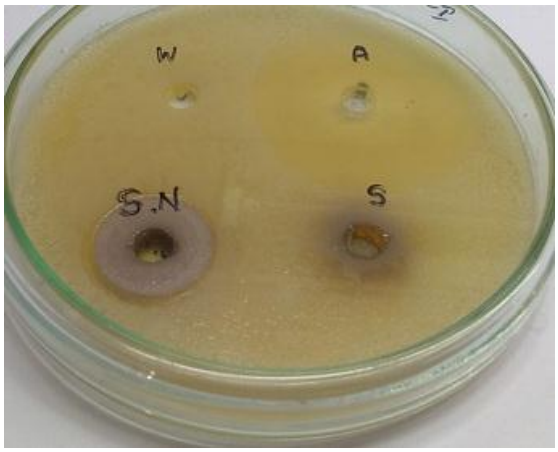
Plate: 1 Phytochemical analysis

1. 200 µg : 2. 400 µg : 3. 600 µg : 4. 800 µg : 5. Positive control : 6. Negative control

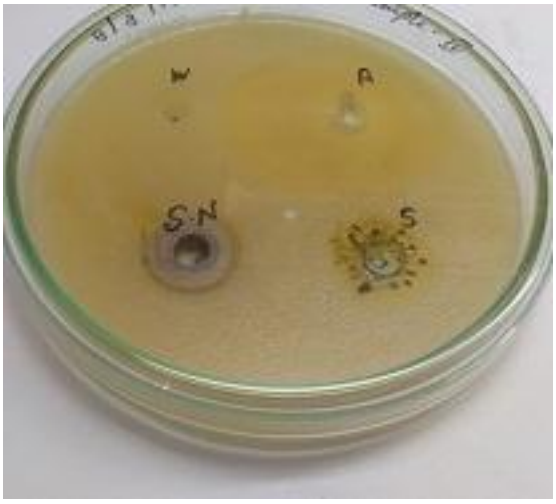
Antibacterial activity of ethanol extract of *Senna alata* leaves was tested against human pathogens *Viz. Strptococcus pneumonia, Enterobacter sp* and *Proteus sp*. *Streptococcus pneumoniae* was highly sensitive to Ethnol extract, the zone of inhibition was 19mm followed by *Enterobacter sp* (17mm), *Proteus sp* (15mm). It is more or less related to

positive control Chloromphenical which exerted the zone of inhibition in the range of 21 mm to 25mm. Table 3; Plate: 2 shows the antibacterial activity of SNPs of *Senna alata* leaf extract on different pathogens.

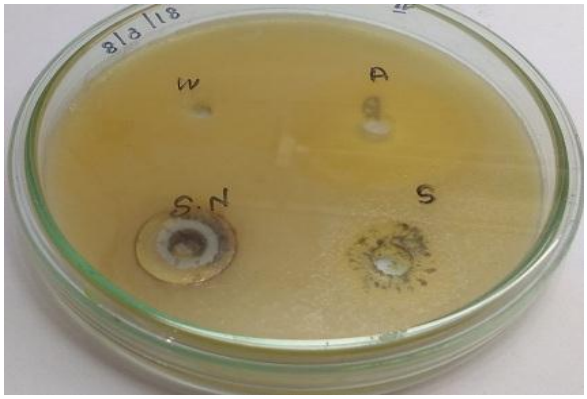
Plate 2: Antibaterial effect of Silver Nano particles



Proteus Sp



Enterobacter



S.Pneumonia

Antibacterial activity of aqueous extracts of *Senna alata* leaves were tested against human pathogens. *Enterobacter sp* was highly sensitive to water extract, the zone of inhibition was 14m.4m followed by *Streptococcus pneumoniae* (13mm), *Proteus sp* (12mm). (Table 3).

Antibacterial activity of SNPs of *Senna alata* leaves were tested against human pathogens. When compared to aqueous and alcohol extracts SNPS showed maximum antibacterial activity. S*treptococcus pneumonia* (21.8mm) showed high degree of susceptibility followed by *Enterobacter* sp (18.3mm) and *Proteus sp* (15.6mm). (Table 4).

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components.[18] The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes. The presence of antibacterial substances in the higher plants is well established. Phytomedicine can be used for the treatment of diseases as it is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug. In recent years, Secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, has been extensively investigated as a source of medicinal agents. It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections.[19]

Current results are supported by the following findings. Cox *et al,*[22] reported that the methanol, and aqueous extract showed highly activity against human pathogens *Strptococcus pneumoniae, E.coli* and *Enterobacter sp.* When the etanolic extract was converted into SNPs and assayed for their antibacterial activity, it was high when compared to alcohol and aqueous alone. *Streptococcus pneumoniae* was highly sensitive to SNPs, the zone of inhibition was 21.8 mm followed by *Enterobacter sp* (18.6mm), *Proteus sp* (15.6 mm). It is more or less related to positive control Chloromphenical which exerted the zone of inhibition in the range of 21 mm to 25mm. (Table 6). Ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity.[20]

The aqueous and chloroform extracts of the *Senna alata, Carica papaya, Cynodon dactylon, Euphorbia hirta, Melia azedarach and Psidium guajava* inhibited the growth of the tested microorganisms viz., *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus Streptococcus pneumonia, Enterobacter sp* and *Proteus sp* showing that the extract contains substances that can inhibit the growth of some microorganisms.[21]

Other workers have also shown that extracts of *Senna alata* inhibited the growth of various microorganisms at different concentrations. The observed antibacterial effects on the isolates is believed to be due to the presence of alkaloids, tannins and flavonoids which have been shown to posses antibacterial properties.[22] The passage of the active compound through the gram negative cell wall may be inhibited. It is though that observed differences may result from the doses used in this study. In addition, microorganisms show variable sensit*ivity* to chemical substances related to different resistance levels between strains.[23]

Recent works have highlighted the role of polyphenolic compounds of the higher plants such as flavonols, anthaquines effetive against *Sterpoccus pneumonia, Enterobacter sp* and *Proteus sp.*.Various scientific studies reported the analgesic, anticancer, antiviral, antimalarial, antibacterial, and antifungal, antifeedent and antifertility activity of this plant.[24]

With the current investigation, it is lime lighted that, when compared to water extract alcohol extract of *Senna alata* was effective in controlling *Sterptococcus pneumoniae, Enterobacter sp* and *Proteus sp.* In addition, if the extracts are converted into SNPs by green synthesis the penetration of SNPs might be high inside the pathogens, hence their antibacterial activity was observed in quiet high range. Moreover the phytochemicals of *Senna alata* was also played a major role after the SNPs penetration inside the cells. On further analysis, from this *Senna alata* we could find a safe, natural antibacterial agent to combate the diseases caused by *Sterptococcus pneumoniae, Enterobacter sp* and *Proteus sp.*

In the present investigation common human pathogens such as *Streptococcus pneumonia, Proteus. Sp*and *Enterobacterssp*which are the causative agents of respiratory tract and urinary tract infections were selected and assayed for their susceptibility for *Senna alata* leaves extract. (Plate:1). Phytochemical profiling of the plant extract was also carried out.

The preliminary phytochemical analysis was carried out for the aqueous and alcohol extract of *Senna alata*leaves. The phytochemical analysis was carried out in the two different extract. The qualitative analysis of the ethanolic and water extracts of *Senna alata*revealed the presence of alkaloid, flavanoid, The water extract of *Senna alata*containstannin and phenolic compound. Moreover, the highest yield was also observed in alcohol extract which includes alkaloids, flavonoids, coumarins, saponin, phenol, tannin, glycosides, and steroids, hence this was selected for further studies (Table1). The leaves are reported to be useful in treating convulsion, gonorrhoea, heart failure, abdominal pains, oedema and is also used as a purgative.[9]

Other scientific names of Cassia alata are Senna alata, Herpetic alata and Cassia bracteata[25] (Abubakar et al., 2008). Cassia alata, is a herb commonly used in Nigeria for the treatment of ringworm, eczema etc. Some of the local Nigerian names are Ilesko and Rinji in Yoruba and Hausa respectively.[5] Traditionally, the leaves are pounded and rubbed on the skin to cure skin diseases.[26]

Many reports have shown that some Cassia species contain antimicrobial substances, particularly Cassia alata.[27] Recent studies revealed that Cassia alata has been proven to be effective against bacteria and fungi species.[3] They observed that the minimal inhibitory concentration (MIC) values of methanolic extracts of the leaves of Cassia alata against Staphylococcus aureus and Bacillus subtilis were 10mg/ml and 2.5 mg/ml respectively. It has been observed that antimicrobial activity of the plants is associated with the presence of some chemical components such as phenols, tannis, saponins, alkaloids, steroids, flavonoids and carbohydrates (Ref). Pieme *et al.*,.[26] have investigated the antifungal and antibacterial activity of Cassia alata and have got positive results. This study is aimed at investigating the antimicrobial activity of the root and leaf extracts of the Senna alata plant against some infectious bacteria and fungi as well as determining the physiochemical and microbiological quality of the plant.

Our findings are supported by the following researches. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. The development of microbial resistance to antibiotics has led the researches to investigate the alternative sources for the treatment of resistant strains. Phytochemical analyses revealed that *Senna alata*

contained saponins, alkaloids, flavonoids, tannins, phenols and glycosides as shown in Table1.

Flavonoids and other physiochemical constitute of the plant also have antimicrobial properties and this is in agreement with Singh and Bhat, 2003 which reported that flavonoids are responsible for the antimicrobial activity associated with some ethnomedicinal plants which. Plant essential or volatile oils and their individual components have been used in traditional systems of medicines for a variety of bacterial infections for centuries and this is also in agreement with Odebiyi,[28] Furthermore, it has been demonstrated that antibacterial properties of these oils can be attributed to their hydrocarbon and terpene constituents. The presence of glycosides and alkaloids in Musa sapientum peels may be attributed to their use by traditional medicine practitioners in healthcare systems in the treatment of some bacterial infections such as cough, fever, cold and venereal diseases.[5]

The results of this research highlights the fact that the organic solvent (ethanol) extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted more or only through the organic solvent medium. This observation agrees with the report of other investigators of medicinal plants that organic solvents are more suitable for extraction of phytochemicals.[29] Different solvents have different solubility capacities for different phytoconstituents, hence the differences in the activities of the various extracts. These bioactive compounds have been reported to be used by plants for protection against bacterial, fungal and pesticidal infections and are responsible for antimicrobial activity.[30] Pieme *et al.*,.[26] investigated the antifungal and antibacterial activity of Cassia alata and got positive results.

The result of phytochemicals in the present investigation showed that the plant leaves contain components like alkaloids, phenols, saponin, Tannins and coumarin. This study reports the presence of different phytochemicals with biological activity that can be valuable therapeutic index.[31][32] In the present study, we have found that the biologically active phytochemicals were present in the methanolic extracts of few medicinal plants. The antimicrobial properties of these extracts may be due to the presence of above mentioned phytochemicals.

CONCLUSION

It may be concluded from this study that *Senna alata* leaf extract has antimicrobial activity against pathogens. Phytochemical and antimicrobial activity of *Senna alata* extract showed

that it is mainly due totannins, reducing sugar, glycosides and triterpenes. Thus this plant could be utilized as an alternative source of useful antimicrobial drugs. Despite the significant progress made in microbiology and the control of microorganisms, sporadic incidents of epidemics due to drug resistant microorganisms pose an enormous threat to public health. we could find a safe, natural antibacterial agent to combat the diseases caused by *Sterptococcus pneumoniae*, *Enterobacter sp* and *Proteus sp.* In addition to that, with the assistance of biotechnology, clinical research and bioinformatics, we would definitely find an alternate safe drug candidate from this *Senna alata* leaves extract. We believe that the result of this study will be an encouragement for further study that will lead to the use of the active components of Senna alata in drug preparation in the nearest future.

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