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Phytochemical Determination and Antimicrobial Investigation of some Selected Indian Medicinal Plants

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Abstract: *Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. Recently, there has been growing interest in the traditional cures of livestock diseases become of expensiveness of pharmaceutical products. The self-help approaches inform of traditional medicines, especially from medicinal plants offer a way by making use of the study was to find out the bioactive chemical constituents and to evaluate the antimicrobial activity of the methanolic extract of traditionally used five medicinal plant of India. A qualitative phytochemical analysis was performed for the detection of alkaloids, flavonoids, saponin and tannins, each analysis was carried out in triplicate which shows the positive result for alkaloids, flavonoids, saponin and tannins respectively, the highest yield of methanolic extract was found in Daturastramonium and lantana camera in the alkaloid fraction. The antibacterial effect of some selected Indian medicinal plants was evaluated on bacterial strains like Staphylococcus species, Escherichia coli species and Bacillus species. The invitro antimicrobial activity was performed by disc diffusion method. The methanolic extract of Daturastramonium and Lantana camara showed the maximum activity against Staphylococcus species in alkaloids fraction compare to the flavonoid and saponin. The Daturastramonium Showed the moderate zone of inhibition against the Escherichia coli species and Bacillus species. The flavonoid extract of the lantana camara and daturastramonium showed the moderate zone of inhibition and the saponin showed the minimum zone of inhibition. However this work highlights its antibacterial sensitivity in par modern standard antibiotics. The result is quite encouraging, further studies needed.*

Keywords: *Medicinal plants, Phytochemicals, alkaloid, flavonoid, saponin, tannin, human pathogens, antimicrobial activity.*

1. INTRODUCTION

In developing countries microorganism are frequently causes of prevailing diseases**,** presenting a serious public health issue in a significant segment of the population as uncovered by either private or official health care system(A.Rekha et.al 2011). Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies [Gloria E.Barboza et al., 2009]. In the recent years there has been an increasing awareness about the importance of medicinal plants [Dewick, P.M. et al., 1996]. According to WHO medicinal plants would be the best source to obtain variety of drugs. Phytochemical which possess many ecological and physiological roles are widely distributed as plant constituents [Michael Wink et al., 2010] .The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoingepidermidis of human immunodeficiency virus (HIV) infections (Dean DA and Gonalez 1996). This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants (Cordell GA.et al, . 2000). Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections (Sieradzki K, et al, .1999). Plant extracts have been used for centuries as a popular method for treating several health disorders. Numerous studies have been carried out on various natural products screening their antimicrobial activity. In the present work, qualitative phytochemical analysis was carried out in Five plants [Abutilonindicum, Adathodavisca, Daturastramonium, Lantana camara and tridax procumbent]for the antimicrobial activity Which have been known to possess medicinal property.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification:-

The Plant materials Adathodavisca, Daturastramonium, Lantana camara, tridaxprocumbens, and Abutilonindicum were collected from fields in and around Bangalore city.

2. 2 Preparation of Plant Material:-

The leaves plucked from the plant were washed 2-3 times with running tap water and was then air dried under shade. After complete shade drying the leaf material was ground in the mixer to obtain the powder and stored in plastic bags.

2.3 Extraction of Plant Material

Preparation of aqueous extracts:

Powdered leaf was homogenized with 5 gm of plant material was in 25 ml of water and the suspension was heat to 50-60 ˚c, and maintain for 15 minutes and then filtered. The filtrate was then centrifuged at 2500 rpm for 15 minutes and the clear supernatant was stored at 5° C until use.

2.4 Phytochemical Analysis

The phytochemical analyses were carried out according to the standard methods with minor modification. Phytochemicals analysis of the crude powder of the Adathodavisca, Daturastramonium, Lantana camara, tridaxprocumbens, and Abutilon indicum, for the tests of phytochemicals as a alkaloid, saponin, tannins, flavonoides and protein etc were made as shown below.

2.4.1 Test for Alkaloides

200 mg plant materials were taken and added 10 ml Methanol and then filtered. After that 2 ml filtrate were taken and added 1 % HCL with steam 1 ml filtrate and 6 drops Mayer´s reagent/Wagnersreagent/ Dragendorffsreagent.It produced creamish/Brown /Orange precipitate indicate the presence of alkaloids.

2.4.2 Test for Saponins

Approximate 0.5 ml filtered were taken and added 5 ml distilled water. Frothing persistence indicate presence of Saponin [GA Ayoola, HAB Coker].

2.4.3 Test for Tannins

200 mg plant material were taken and added 10 ml distilled water and then filtered. After that 2 ml filtered were taken and added 2 ml $FeCl_3$ Blue. Then black precipitate indicate the presence of Tannins & Phenols.

2.4.4 Test for Flavonoides

200 mg plant material were taken and added 10 ml Ethanol, then Tomato, Red colour indicate the presence of Flavonoids.

3. SELECTION OF BACTERIAL STRAINS

The investigated microorganisms consisted of Two Gram-positive bacteria: Staphylococcus aureus ATCC25923, Bacillussubtilis TCC6633; one Gram-negative bacteria: Escherichia coli ATCC25922. Microorganisms were obtained from the National Chemical Laboratory (NCL), Pune, India. Microorganisms were maintained at 4 °C on nutrient agar slants.

3.1 Antimicrobial susceptibility test

The antimicrobial assay was performed by agar disc diffusion method (Bauer AW, et al, . 1966). The 20ml of sterilized Muller Hinton Agar was poured into sterile petriplates, after solidification, 100 µl of fresh bacterial culture were swabbed on the respective plates. Each of discs which are approximately 5mm in diameter was cut from Whatman filter paper.The sterile discs were kept over the agar plates using sterile forceps at various concentration (2, 4, 6, 8, and 10µl). The plates were incubated for 24 hours at 37 ˚c. After incubation the diameter of inhibitory zones formed around each discs were measured (mm) recorded. (Nair R, KalariyaT, et al, .2005).

Table 1: Phytochemical Analysis of Crude Extract from the Leaves of Five Medicinal Plants

SL. NO	Phytoconstituents	Adathodavisca	Abutilon indicum	Daturastramonium,	Lantana camara	Tridaxprocumbens
1	ALKALOIDS (a)Mayer's test (b)Dragndore's test (c)Wagner's test	+ + +	+ + +	+ + +	+ + +	+ + +
2	FLANOIDS (a)Shinoda test (b)Alkline reagent (c)FeCl3 test	+ + +	+ + +	+ + +	+ + +	+ + +
3	TANNINS (a)Lead acetate test (b)FeCl3 test	+ +	+ +	+ +	+ +	+ +
4	SAPONINS (a)Frathing test	+	+	+	+	+
5	AMINOACID (a)Millons test (b)Ninhydrin test	– –	– –	– –	– –	– –
6	PROTEIN (a)Biuret test (b)Millons test	– –	– –	– –	– –	– –

+[Present] , -Absent]

4. RESULT

The phytochemical analysis of the crude extract of the leaves of five medicinal plants revealed the presence of only alkaloids, flavonoids, tannin and saponin of all the phytochemical screened for anthraqinones, aminoacid and protein were found to be absent (Table 1). The result obtained for the antimicrobial test performed on different extract of medicinal plants and zone of inhibition of the individual plant extract with three type of bacterial pathogen were identified that is Escherichia coli, Bacillus subtitles, Staphylococcus aureas.

Alkaloids antibacterial susceptibility test of five medicinal plants against some species of bacteria.

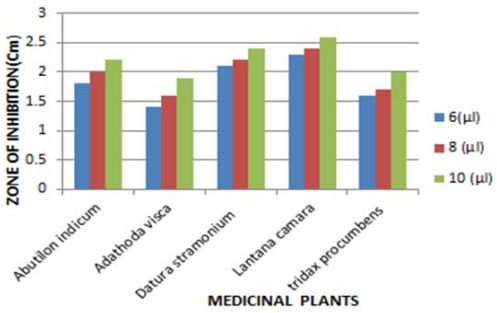

Fig. 1: Escherichia Coli

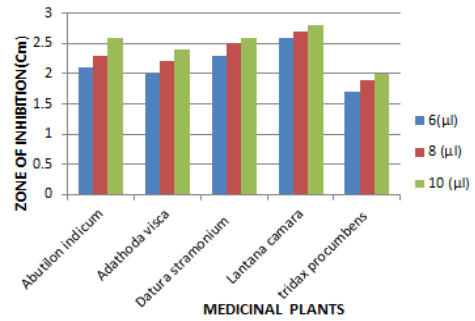

Fig. 2: Staphylococcusaureus

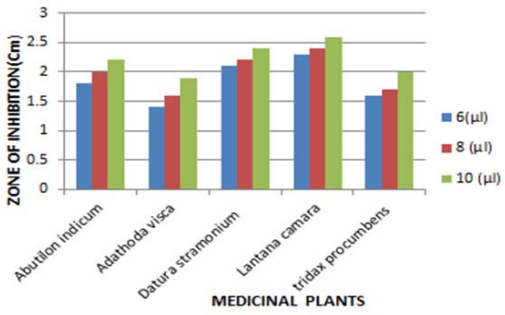

Fig. 3: Bacillus Subtitles

Fig. 1: Represents the alkaloids antibacterial susceptibility test results for Escherichia coli , Daturastramonium and Lantana camara show the significance zone of inhibition that is the higest zone inhibition 2.0 cm was noted in the concentration of 10µl.Adathoda visca and tridaxprocumbens 1.6cm was noted in 10µl. **Fig. 2**: Represents the alkaloids antibacterial susceptibility test results for Staphylococcus aureas, Lantanacamara show the significance zone of inhibition 2.8cm was noted in the concentration of 10 µl and 2.7cm noted in the 8 µl and 2.6 for 6 µl . Daturastramonium is the second highest zone of inhibition 2.6cm was noted in the 10 µl.

Fig. 3: Represents the alkaloids antibacterial susceptibility test results for Bacillus subtitles.For Lantana camara, the highest zone of inhibition 2.6 cm was noted in the concentration of 10 µl, Datura stramonium, 2.4 cm was noted in the concentration of 10 µl, Abutilon indicum, 2.2 cm concentration of 10 µl, adathodavisca, 1.9 cm concentration of 10 µl, Tridaxprocumbens 1.8 cm concentration of 10 µl. From the above observation Lantana camara showed the significance zone of inhibition against the Bacillus subtitles. 1.1 cm in 8 µl and 1.2 cm was noted in the concentration of 10 µl.From the above observation Lantana camara showed the significance zone of inhibition against the Escherichia coli.

5. DISCUSSION

The increasing trend of the resistance to the antibiotics in current use has drawn the attention of researcher to natural alternative treatment of bacterial infection as potential source of new novel antibacterial agent.This study indicated that all the five plant extract were showing the antibacterial activity in different inhibition.The gram negative bacteria are reported to be resistant against most antibacterial against most antibacterial agents as a result of the more complicated nature of their cell wall compared to gram positive bacteria (Akinyemi et al., 2005; Goyal et al., 2008).However the five species were found to be active against the two grope of bacteria underlining their ethno medicinal use for treatment of various infection diseases. The present study is an attempt to evaluate plans as source of potential chemotherapeutic agents and antimicrobial activity (cledson.V, et at, .2007). The search of novel bioactive compounds including antimicrobial ones continues. This is largely so because some pathogens have developed resistance to certain currently used drugs and some disease have yet to be treated chemotherapeutically (chin .Y, et al, .2006). Medicinal plants are necessity for the scientific point of view, to establish a rational relationship between chemical biological and therapeutical activities of folklore medicine (Harborne J.B, 1973).Our study stated that the antimicrobial activities of five medicinal plants (Abutilon indicum, Adathodavisca, Daturastrarmonium , Lantana camara , tridaxprocumbens) due to the presence of the following phytochemicals namely, alkaloids, flavonoids, Saponins and Tannins. The potential of antimicrobial properties of plants are related to their ability to synthesize compounds by the

secondary metabolism (Clark AM, et al, .1993). It has also been shown that tannins are biologically active, against E. coli, S. aureus and Bacillus subtilius. Results obtained from this study, indicated that, the plant extracts showed the strongest antimicrobial activity than the commercially available antibiotics. The antimicrobial activity of methanolic extract of Daturastarmonium and lantana camara showed the signifince antimicrobial activity various phytopathogens.

6. CONCLUSION

The preliminary phytochemical screening and antimicrobial activity of five medicinal plants studied here can be seen as the potential source of useful drugs. Different extract showed varying degrees of antibacterial activities against microbes tested here. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent and we therefore, suggest further, the purification and characterization of the phytochemicals that would be obtained with a view to obtaining useful chemotherapeutic agent. The plant studied here can be a source of high pharmacological importance and potential source of new drugs. Further studies on such bioactive compounds screening and their antimicrobial activity will unravel the potentiality of these traditional medicines.

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