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STUDIES ON THE PHYTOCHEMICAL CONSTITUENT AND ANTIMICROBIAL POTENTIAL OF AN IMPORTANT MEDICINAL PLANTS: *GYMNEMA SYLVESTRE* (RETZ.) R. BR. EX SM

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Abstract

The traditional system of medicine utilizes the potentiality of herbal medicinal plants as these are served as reservoir pool of various herbal drugs and due to this, Ayurveda (the oldest medicinal system) is accepted globally. The growth of herbal industrial sector completely depends on the medicinal plants which are served as potent source of the medicinal drugs. Since the prehistoric time these medicinal plants are used as the source of valuable drugs. The plant based therapeutic agents are safe and have no side effect, hence widely used as medicinal drugs. The use of these herbal medicines gaining momentum throughout the world. These are the phytochemicals on which medicinal property of plant depends and they also plays active role in the rejuvenation of body system. These bioactive compounds of the medicinal plants are the source of medicinal drugs. Due to bacterial and fungal infection many types of disease are created and agricultural sector adversely affected. Various herbal plants has the antimicrobial properties. Therefore the present investigation is focused on the evaluation of antimicrobial action of *Gymnema sylvestre* plant. It was found that leaf extract of this plant have the strong antibacterial properties against the test bacterial strain. It was also observed that leaf extract of this plant inhibited the conidial germination of all the test fungal pathogens. Different types of secondary metabolites are obtained from the leaf extract that served as potent tool against bacterial and fungal pathogens.

Key words : Ayurveda, herbal medicines, phytochemicals, antibacterial, antifungal properties, *Gymnema sylvestre.*

Introduction

The potential of medicinal plants, able them to serve as a reservoir pool in traditional system of medicines as a grist for herbal drugs. Since eons, medicinal plants are used as the stock for uncountable and valuable drugs. The valuable repository of pharmaceutical intermediates and bioactive compounds of medicinal plants are source of medicinal drugs and plays a huge role in the construction of traditional systems such as Ayurveda, Unani, Siddha, Chinese and Tibetan all over the world. The bioactive chemicals executed an implacable personification to sustain and resist the plants under the biotic and abiotic stresses (Treutter, 2006, Manonmani *et al.*, 2009).

Diseases are the deficiency caused by mis-metabolism of body (genetic errors) or external factors like biotic (bacteria, fungi) and abiotic (UV-radiation, pollution) agents. Chemical medicines, allopathy suppress the disease in place of filling the shortcomings. Investigation on the phytoconstituents of plants is an interesting area of research as medicinal plants synthesize a vast variety of secondary metabolites that are useful for the life of human being. These secondary metabolites that played a vital role in controlling diseases and act as both prophylactic and therapeutic protective shield for the maintenance and preservation of human health (Upadhyay *et al.*, 2014; Ansari and Inamdar, 2009; Menghai *et al.*, 2010,Onsare *et al.*, 2013; Teiten *et al.*, 2013; Aumeeruddy-Elalfi *et al.*, 2016). Wisdom about the uses and benefits of plants derived chemicals and drugs are also mentioned in ancient literatures like Atharveda, Charak Samhita, Sushruta Samhita. In fact, a statue of surgeon Sushruta is placed in Royal Australia College of Surgeons, Melbourne of Australia delineating

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the acceptance and increase of eco-friendly therapeutics.

Phytochemicals of medicinal plants are institutions for herbal medicinal drugs and also plays an active role in the rejuvenation of body system. An impressive alternate of allopathy and homeopathy is provided by the phytochemical principles of the medicinal plants in the therapeutics and due to the safety and little to no side effect influences human beings to entrust herbal medicines for improving their health related issues (Banerjee *et al.,* 2012; Singh and Dubey, 2012; Rawat *et al.,* 2010).

India is notified as one of the richest center of biodiversity. A several thousand plant species are listed in the Indian flora, many of them have recognized to produces bioactive compounds that are pharmacologically relevant (Shakya, 2016; Cushnie *et al.,* 2014). The applicability of herbal medicines now gaining incitation due to their better effectiveness and the life protective drug formulation in comparison to the synthetic or marketed drugs (Chashoo *et al.,* 2012).

Antibiotic potential of plants shows varying degree of hypoglycaemic and antihyperglycemic activity (Rahimi, 2015), also contribute to the herbal medicines in gaining momentum throughout the world. All these reasons makes the Ayurveda, the oldest medicine system accepted globally and makes herbal dugs an entirely different and big industrially sector, that depends on the flora, especially medicinal plants which served as a potent source of the herbal medicinal drugs. Department of Ayush (Drug Control Cell) run by Ministry of Health and Family Welfare of Government of India published a list of two hundred seventy seven herbal medicines in Essential Drugs List (EDl), includes medicines like Pancha-Valkala Kashaya Churna, Brahmi Ghrita and many more.

Not only human body or livestock but biotic and abiotic agents effects feed and fodder too. During storage, the grains and foodstuff destroyed by the activity of fungal pathogens. The nutritive values of storage products decreased due to mycotoxins production by fungi (Mannaa and Kim, 2017). Every year in the world, due to contamination by mycotoxins a large proportion of foodstuffs become unhealthy for the consumption of living being (Atanda *et al.,* 2011; Adeyeye, 2016). These mycotoxins create life threatening disorders as these are hepatotoxic, carcinogenic and nephrotoxic (Zain, 2011). Herbal fungicides because of their eco-friendly nature are gaining growing interest for control of fungal pathogens (Srivastava and Singh, 2011) in eco-friendly, no side effect way.

Therefore essential practices are taken into consideration for the destruction of bacterial and fungal activities. The plants have the various types of antibacterial and antifungal activities in the form of active chemical constituents that significantly retarded the survival and germination of bacterial and fungal spores.

Gymnema sylvestre R. Br., mostly found in India, commonly called as Gurmar (sugar destroyer), also known as "miracle fruit". Bioactive properties such as antimicrobial, anticancerous, antioxidants, and hypolipidemic activities present in this plant (Laha and Paul, 2019).

Keeping this view in the mind the present investigation based on the study of phytochemical constituents and antimicrobial properties of *Gymnema sylvestre* (Retz.) R. Br. ex Sm.

Materials and Methods

Collection of plant material and preparation of plant extract

The plant material was collected from the local nurseries. The leaves of plant after washing were shade dried and powdered in mechanical grinder. The extract of dried leaves sample of plant were prepared in ethanol and methanol at room temperature by simple extraction method. The 50 g dried powder of leaves sample was mixed with 200 ml solvent in 500 ml conical flasks. The flasks were plugged tightly with cotton and wrapped with paper. All conical flasks were kept on shaker for 24 hours, and then allowed to stand for 5 hours to settle the plant materials. Thereafter the collected solutions were filtered through Whatman No.1 filter paper. The supernatant was collected and the extract were evaporated at 45^0C in vacuum evaporator to make the final volume $1/5^{th}$ of the original volume and then stored at 4^0C in air tight bottles.

Qualitative analysis of phytochemical constituents

The freshly prepared methanolic and ethanolic leaf extracts were analysed to find out the different phytochemicals by using the standard procedures.

Alkaloids

Dragendroff's reagent/ Mayer's reagent/ Wagner's reagents were used to detect the presence of alkaloids. The appearance of orange / yellow /brown colour indicated the alkaloids.

Anthraquinones (Bomtregers test)

The plant extract (2-5 g) was mixed with 10 ml of benzene. After filtration, 5 ml of 10% ammonia solution was then added to the filtrate, the presence of violet colour indicated the anthraquinones.

Cardiac glycosides (Keller-Killani test)

The 2 ml glacial acetic acid (containing one drop of

ferric chloride) solution was added to the 5 ml of methanolic and ethanolic extract. Appearance of brown coloured ring indicated the presence of cardiac glycosides.

Flavonoids

A 5 ml of dilute ammonia solution was added to the aqueous filtrate of each leaf extract, after the addition of 2 ml of concentrated sulpuric acid, the yellow color appearance indicated the presence of flavonoids.

Phenols

A few drops of neutral ferric chloride solution were added to the solvent plant extract, appearance of intense colouration indicated the presence of phenols.

Saponins

2 g of the powdered leaf sample + boiled distilled water (20 ml). A 10 ml of the filtrate + 5 ml distilled water, 3 drops of olive oil was then added; formation of emulsion indicated the presence of saponins.

Steroids

Alcoholic extract + LibermannBuchard Reagent '! violet colour indicated the presence of steroid.

Test for tannins

Plant powder + $FeCl_3$'!blue-black coloration indicated the presence of tannins.

Terpenoids (Salkowski test)

The two ml chloroform + 3ml of concentrated sulphuric acid was added to 5 ml of each extract '! a reddish brown coloration indicated the presence of terpenoids.

Antibacterial Activity

Agar well diffusion bioassay was followed to determine the antibacterial activity of the *Gymnema* leaf extract. An appropriate amount of saline suspension (diluted in sterilized NaOH) of pure culture of the given two bacterial slant was added to molten test agar. The 500 µl of this culture was mixed into 18 ml of sterile molten cool nutrient agar medium, mixed well and poured into sterile Petri dishes (90 mm). The Petri dishes so prepared were allowed to solidify and were marked with the organisms inoculated. Thus, in all 6 Petri dishes: 3 Petri dishes for *Escherichia coli* and 3 petri dishes for *Staphylococcus aureus* mixed with medium. Now in each Petri dishes a 6mm diameter well of *E. coli* and in another 5 petri dishes a 6mm diameter well of *S. Aureus* was placed with the help of sterilized cork-borer. In each Petri dish 30 µl of leaf extract was added to one well, 60 µl to another well and 90 µl to the third well. The well with only distilled water served as control. All these Petri dishes were incubated overnight at 37^0C. The Inhibition zone, if any, was measured using a fine mm scale and expressed as distance (in mm) from edge of the well to the normal colony growth of the test bacteria.

Antifungal activity

The poisoned-food technique was used to determine the antifungal activity of leaf extract (methanolic and ethanolic) of the plant against three test fungi.The 10 ml, 20 ml, 30 ml and 40 ml of each extract were added to eight separate flasks (4 flasks for methanolic and 4 flasks for ethanolic extracts) containing pre sterilized but warm PDA (potato dextrose agar medium) in such a manner that the total volume (extract + PDA) was equals to 100 ml thus yielding final concentrations of 0.10%, 0.20%, 0.30%, and 0.40% of the extract in the culture media respectively. The content of each flasks were poured in eight Petri dishes each (20 ml per Petri dish). All Petri dishes were inoculated centrally with 6mm diameter discs from the seven days old culture of the test fungus. Thus, five sets of eight Petri dishes each were prepared as under for each test fungus:

(a) PDA medium containing 0.10% *Gymnema* leaf extract (methanolic) + test fungus,

(b) PDA medium containing 0.20% *Gymnema* leaf extract (methanolic) + test fungus,

(c) PDA medium containing 0.30% *Gymnema* leaf extract (methanolic) + test fungus,

(d) PDA medium containing 0.40% *Gymnema* leaf extract (methanolic) + test fungus,

(e) PDA medium containing 0.10% *Gymnema* leaf extract (ethanolic) + test fungus,

(f) PDA medium containing 0.20% *Gymnema* leaf extract (ethanolic) + test fungus,

(g) PDA medium containing 0.30% *Gymnema* leaf extract (ethanolic) + test fungus,

(h) PDA medium containing 0.40% *Gymnema* leaf extract (ethanolic) + test fungus,

A set of eight Petri dishes containing only medium served as control. All these Petri dishes were incubated for seven days at 25 ± 1^0C. The diameters of colonies were recorded and the percentage inhibition was calculated using the following formula:

$$\mathbf{I = \left(\frac{E - C}{C}\right) \times 100}$$

Where,

I indicate the inhibition percentage,

C indicates the radial growth of the fungus in control Petri dishes,

E indicates the radial growth of the fungus in the Petri dishes with medium containing the given leaf extracts.

Results and Discussion

During the qualitative analysis of the phytochemicals table 1 it was observed that the alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenols, saponins, and tannins were present in the both methanolic and ethanolic leaf extract of the *Gymnema* plant, but the test conducted for steroids and terpenoids showed the negative results *Gymnema* plant. David and Sudarsanam (2013) also reported similar findings.

A glance from table 2 (Fig. 1) indicated that the methanolic and ethanolic leaf extract of the gurmar plant showed higher antibacterial potential against both the test bacteria. It was noticed that the inhibitory potential increased with the increasing concentration of leaf extract. Sripathi and Sankari (2010) also observed the antibacterial efficiency of methanolic and chloroform extract against *E. coli* and *Proteus* sp., The growth of *E. coli, Staphylococcus aureus,* and *Candida albicans* (Wani*et al.,* 2012) retarded by the leaf extract (methanol and chloroform). Naidu *et al.,* 2013 also observed that the plant leaf extract of *Gymnema* significantly retarded the growth of *S. aureus* and some gram negative bacteria. Murugan and Mohan, 2012 reported that leaf and stem extract (aqueous and petroleum ether) of this plant showed the inhibitory potential against various bacteria *(S. aureus, Bacillus sublitis, Salmonella typhi* and *Klebsiella pneumonia*).

It was observed maximum inhibition of *E. coli* occurred at the 90 µl conc. of both the leaf extract and minimum inhibition occurred at 30 µl conc. of both types of leaf extract studied. Saumendu *et al.,* 2010 and Bhuvaneswari *et al.,* 2011 also reported that the leaf extract of *Gymnema* plant showed antibacterial potential against the bacteria namely, *Bacillus* sp., *E. coli, S. aureus,* and *Pseudomonas* sp. The present investigation clearly indicated that the leaf extract of *Gymnema* plant effectively reduced the bacterial growth. Hence, it can be utilized as promising antibacterial herbal formulation.

Table 3, Fig. 2 indicated that leaf extract of the plant efficiently inhibited the growth of all the test fungi. It was clearly observed that increasing conc. of both type of leaf extract of *Gymnema* plant significantly inhibit the fungal growth. *Candida albicans* causal agent of mucosal and invasive infection of human being greatly inhibited by the gymnemic acids, an important constituent of *Gymnema* leaf (Vediyappan *et al.,* 2013). The conidial germination of both the test fungal pathogens strongly inhibited by metanolic and ethanolic leaf extract of the plant. Vediyappan *et al.,* 2013, also observed the similar results and reported that *Gymnema* leaf extract inhibited the conidial germination of *Aspergillus* species.

Table 1: Qualitative analysis of phytochemical constitutens of *Gymnema sylvestre* leaf extract.

Phytochemicals	*Gymnema sylvestre*	
	Methanolic leaf extract	Ethanolic leaf extract
Alkaloids (Dragendroff's Reagent)	+	+
Alkaloids (Mayer's Reagent)	+	+
Alkaloids (Wagner's Reagent)	+	+
Anthraquinones (Bomtregers test)	+	+
Cardiac glycosides (Keller-Killani test)	+	+
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Steroids (LibermannBuchard Reagent)	—	—
Tannins	+	+
Terpenoids (Salkowski test)	—	—

+ sign. indicated the presence of phytochemical, and –sign. indicated the absence of phytochemical.

Table 2: The zone of inhibition (cms) with different concentrations of leaf extract of *Gymnema sylvsetre.*

Name of bacteria	conc. of leaf extract(methanolic)			conc. of leaf extract(ethanolic)		
	30µl	60µl	90µl	30µl	60µl	90µl
E. coli	1.08	1.52	1.68	1.23	1.34	1.65
S. aureus	1.43	1.51	1.76	1.45	1.61	1.72

Table 3: The radial growth of fungal species in the medium supplemented with different concentration of leaf extract of *Gymnema sylvsetre.*

Fungal species	conc. of leaf (methanolic extract)				conc. of leaf (ethanolic extract)			
	0.10%	0.20%	0.30%	0.40%	0.10%	0.20%	0.30%	0.40%
Aspergillus fumigatus	3.45	2.87	2.87	2.56	4.12	3.64	2.43	2.13
A.niger	3.23	2.75	2.68	2.14	4.56	4.25	2.65	2.21
Candida albicans	3.82	3.67	3.20	1.87	3.72	2.76	2.76	2.36

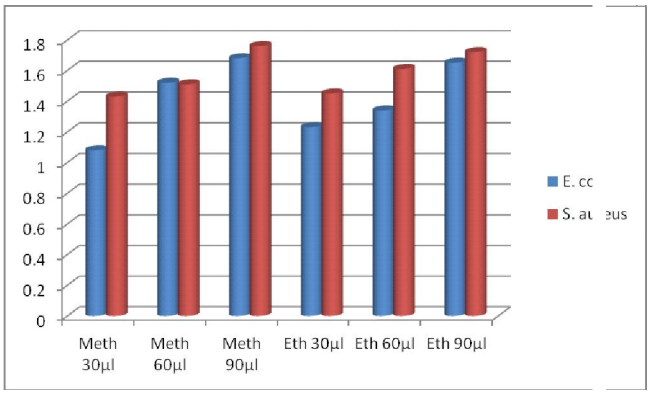

Fig .1: Zone of inhibition at different concentrations of leaf extracts.

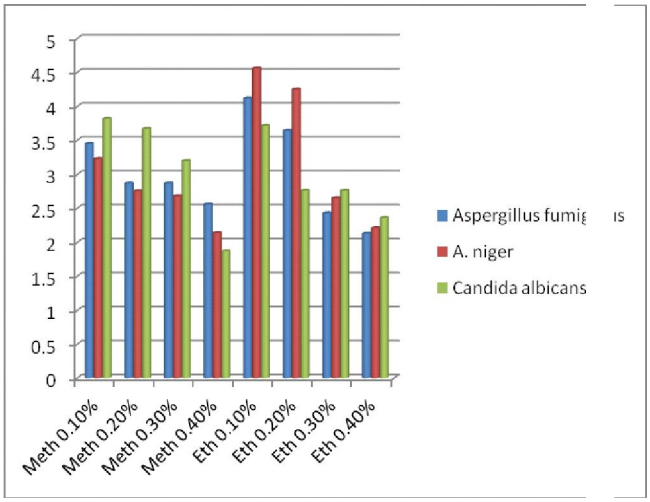

Fig. 2: Inhibiton(%) of conidial germination of test fungal pathogens by leaf extract.

Conclusion

Indian traditional health system based on the herbal formulation and phytochemicals is as old as the human civilization. Therefore the use of herbal medicines gaining momentum and research activities increased in recent years. This study was framed to evaluate the antibacterial and antifungal potential of *Gymnema sylvestre* (Gurmar) which is an important source of wide variety of phytochemical components, and hence, can be utilized as medicine for the treatment of diseases and widely used in the treatment of diabetes mellitus. The methanolic, ethanolic extract, and bioactive chemical constituents exhibited the strong antimicrobial potentiality against test bacterial and fungal pathogens.

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