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ANTIMICROBIAL AND RADICAL SCAVENGING ACTIVITY OF *GYMNOSPORIA MONTANA* (ROTH.) BENTH

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ABSTRACT

Gymnosporia montana (Roth.) Benth. belonging to the family Celastraceae grows in arid and dry areas of India and other countries. In the present study, we determined antimicrobial and radical scavenging efficacy of leaf, flower and fruit extract of *G. montana*. Antibacterial and antifungal activity of extracts was determined by Agar well diffusion and Poisoned food technique respectively. Radical scavenging efficacy of extracts was evaluated by DPPH free radical scavenging assay. Overall, fruit extract displayed marked antimicrobial activity when compared to leaf and flower extracts. Leaf extract scavenged DPPH radicals more effectively than flower and fruit extracts. Fruit extract displayed least radical scavenging potential. In suitable form, the plant can be used to control/treat microbial infections and oxidative stress.

KEY WORDS: *Gymnosporia montana*, Agar well diffusion, Poisoned food technique, DPPH.

INTRODUCTION

Plants have the ability to produce a number of primary and secondary metabolites. The secondary metabolites of plants perform several functions. Most of these plant metabolites possess useful biological and pharmacological properties and are used as chemotherapeutic agents or as ingredients for the development of modern drugs. Chemotherapeutic potential of

plants is due to the presence of metabolites such as phenolics, alkaloids, terpenoids, saponins and others. It is known that over 50% of all modern clinical drugs are from natural origin. Plants are an essential component of traditional medicine and majority of world's population, especially under developing and developing countries, depends on traditional medicine for primary healthcare needs. Ayurveda and other systems of medicine have vast record of medicinal plants being used for the treatment of various types of ailments.[1-7]

Gymnosporia montana (Roth.) Benth. (Celastraceae) is a branched, spinescent shrub or small tree and occurs in arid, dry areas of India and other countries. The plant grows at elevations from near sea level, on the coast on sand, at forest margins, hillsides and on sea cliffs, often on limestone. The plant requires long, hot summers for production of flowers and fruits. The plant grows in moderately fertile, moist but well-drained soil in full sun with midday shade. It is used in indigenous medicine as a cure for various ailments.[8,9] The plant is traditionally used to treat various ailments such as jaundice, ulcers, snake bite, toothache, skin allergy and verm infection.[10-13] *G. montana* is shown to exhibit bioactivities such as hepatoprotective.[11] antimicrobial.[14,15] antioxidant.[16] anti-inflammatory.[17] and analgesic activity.[17] In the present study, we determined antimicrobial and radical scavenging effect of leaf, flower and fruit of *G. montana*.

MATERIALS AND METHODS

Collection and identification of plant

The plant material (*G. montana*) was collected during January 2015 at a place called Jogimatti, near Chitradurga, Karnataka. The plant was authenticated by Prof. D. Rudrappa, Department of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga-01.

Extraction

The leaves, fruits and flowers were separated, washed well using clean water,dried under shade and powdered separately in a blender. 25g of each powdered material was transferred into separate conical flasks containing 100ml of methanol. The flasks were left for two days with occasional stirrings followed by filtering the contents through Whatman No. 1 filter paper. Further, the filtrates were evaporated to dryness and stored in refrigerator until use.[7]

Antibacterial activity of extracts

Agar well diffusion assay was used to assess antibacterial potential of extracts of *G. montana* against three Gram positive (*Staphylococcus aureus*, *Bacillus subtilis* and *B. coagulans*) and

three Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*). The test bacteria were inoculated into sterile Nutrient broth tubes followed by incubation at 37^{o}C for 24 hours. The broth cultures were swabbed over the surface of sterile Nutrient agar plates using sterile cotton swabs followed by punching wells of 8mm diameter using sterile cork borer. 100µl of leaf, fruit and flower extracts (20mg/ml of DMSO [dimethyl sulfoxide, 25%]), chloramphenicol (standard antibiotic, 1mg/ml of sterile distilled water) and DMSO (25%, in sterile water) were transferred aseptically into labeled wells. The plates were left for 30 minutes and then incubated in upright position for 24 hours at 37^{o}C. The zone of inhibition formed around the wells was observed after incubation and measured.[7]

Antifungal activity of extracts *G. montana*

Poisoned food technique was carried out to investigate antifungal activity of extracts of *G. montana* against fungi namely *Bipolaris sorokiniana* (from root rot of wheat), *Fusarium oxysporum* f.sp. *zingiberi* (from rhizome rot of ginger), *Colletotrichum capsici* (from anthracnose of chilli) and *Curvularia* sp. (from mouldy grains of sorghum). In brief, well sporulated cultures of test fungi were inoculated on control (without extract) and poisoned potato dextrose agar plates (1mg extract/ml of medium) by point inoculation method using sterile inoculation needle. The plates were incubated for 5 days at room temperature in upright position. Later, the diameter of fungal colonies on both control and poisoned plates was measured in mutual perpendicular directions. Antifungal activity of extracts, in terms of inhibition of mycelial growth (%) of test fungi, was determined using the formula: Inhibition of mycelial growth (%) = (C – T / C) x 100, where C and T refers to diameter of fungal colonies on control and poisoned plates respectively.[7]

Radical scavenging activity of extracts of *G. montana*

In order to evaluate radical scavenging potential of extracts, we conducted DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging assay. In brief, 1ml of different concentrations of leaf, fruit and flower extracts (6.25-100µg/ml) was mixed with 3ml of DPPH solution (0.004% in methanol) in clean and labeled tubes. The test tubes were incubated at room temperature in dark for 30 minutes followed by measuring the absorbance at 520nm. The absorbance of DPPH control (1ml methanol+3ml DPPH) was noted. The radical scavenging activity (%) of each concentration of extracts was calculated using the formula:

Scavenging activity (%) = (Ac–At/Ac)x100, where Ac and At refers to absorbance of DPPH control and DPPH and extract/standard combination respectively. Ascorbic acid was used as reference standard.[7] IC_{50} value for each extract was calculated which indicates the concentration of extract required to scavenge 50% of DPPH free radicals.

RESULTS AND DISCUSSION

Antibacterial activity of extracts of *G. montana*

Antibiotic resistance is a global problem. Improper use of antibiotics resulted in the emergence of antibiotic resistant bacteria which made the therapy most difficult. Moreover, the ability of these bacteria to spread the resistance genes to susceptible bacteria has made the situation even worst. Due to this, an immense interest has been devoted to natural products, in particular plants, for the discovery and development of novel antimicrobials. Plants have been exploited for the development of bioactive agents with activity against dreadful pathogens.[1,7] In the present study, we evaluated antibacterial activity of leaf, flower and fruit extract of *G. montana*. The result of antibacterial effect of extracts from different parts of *G.montana* is shown in Table 1. In case of *E.coli*, fruit extract exhibited high inhibitory activity followed by flower and leaf extract. *S.typhi* was inhibited to higher extent by fruit extract followed by leaf and flower extracts which showed similar inhibitory effect. *S.aureus* was inhibited to higher extent by fruit extract followed by leaf and flower extracts. Inhibition of *B.subtilis* was highest in case of leaf extract. *B.coagulans* was inhibited to more extent by flower and fruit extracts. Flower extract was shown to cause high inhibition of *P.aeruginosa*. Over all, fruit extract displayed marked inhibitory activity against *S. aureus, E. coli, B. coagulans* and *S. typhi* when compared to other extracts. *P.aeruginosa* was inhibited to high extent by leaf and flower extract. Reference antibiotic caused marked inhibition of test bacteria. There was no inhibition of test bacteria in case of DMSO. In an earlier study, aqueous extract from stem of *G. montana* displayed high inhibition of bacteria when compared to other solvent extracts and the inhibition observed was concentration dependent.[18] In another study, Moteriya *et al*.[14] showed inhibitory potential of leaf and stem extracts of *G.montana* against Gram positive and Gram negative bacteria.

Table 1: Antibacterial activity of extracts of *G. montana*

Test bacteria	Zone of inhibition in cm				
	Leaf extract	Fruit extract	Flower extract	Antibiotic	DMSO
S. aureus	1.4	2.5	1.3	2.5	0.0
E. coli	1.5	2.5	2.0	3.0	0.0
P. aeruginosa	2.0	1.6	2.3	2.1	0.0
B. coagulans	1.5	1.7	1.7	3.0	0.0
B. subtilis	1.8	1.5	1.6	2.9	0.0
S. typhi	1.5	1.6	1.5	2.9	0.0

Antifungal activity of extracts of *G. montana*

Like antibiotics, indiscriminate use of synthetic chemicals for the control of plant pathogens is disadvantageous. It leads to environmental pollution, adverse effect on human and animal health and also emergence of resistant pathogens. Hence, biological control is an important approach to control phytopathogenic fungi. It is an ecofriendly approach used for plant protection and it reduces problems associated with synthetic agents,[7,19,20] The result of antifungal effect of extracts from different parts of *G.montana* is shown in Table 2 and Figure 1. The extracts were shown to inhibit all test fungi but to varied extent. Fruit extract displayed stronger antifungal effect against all test fungi when compared to other extracts. *B.sorokiniana* was inhibited to high extent by fruit extract followed by flower and leaf extracts. *Curvularia sp*. was more susceptible to fruit extract followed by leaf and flower extracts. Inhibition of *C.capsici* was marked in case of fruit extract followed by leaf and flower extracts. *F.oxysporum* was susceptible to higher extent by fruit extract followed by flower and leaf extracts. In a previous study, Singh and Vidyasagar,[15] observed inhibitory potential of ethyl acetate and methanol extracts of *G. montana* against dermatophyte *Microsporum gypseum*.

Table 2: Colony diameter of test fungi on control and poisoned plates.

Test fungi	Colony diameter in cm			
	Control	Leaf extract	Fruit extract	Flower extract
Curvularia sp.	4.2	2.6	2.2	3.0
B. sorokiniana	3.9	2.7	1.5	2.0
F. oxysporum	4.8	3.3	2.6	3.0
C. capsici	4.2	3.2	2.4	3.3

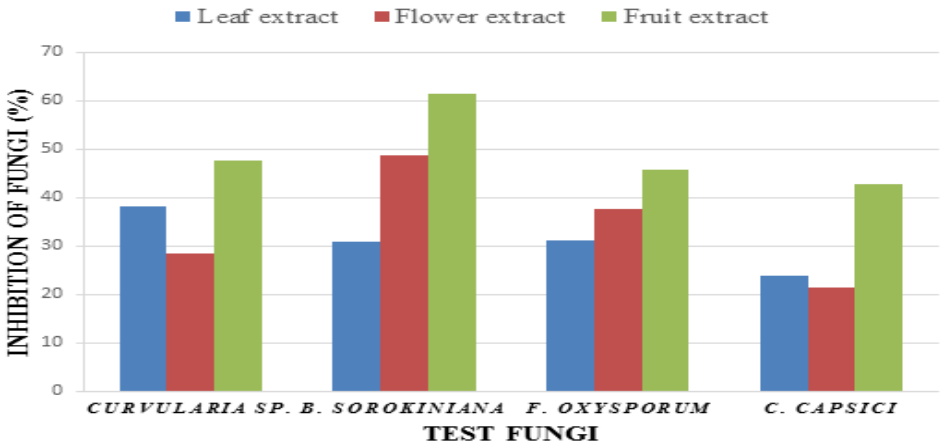

Figure 1: Inhibition (%) of test fungi by extracts of *G. Montana.*

Radical scavenging activity of extracts of *G. montana*

Oxidative stress induced by free radicals causes a number of diseases or disorders such as aging, cardiovascular diseases and neurodegenerative diseases in human beings. Negative effects of synthetic antioxidants triggered immense interest in finding antioxidants from natural sources. Plants are known to be one of the richest reservoirs of antioxidant agents. There are many methods being used for determining radical scavenging activity. Among these, the DPPH scavenging assay is one of the widely used in vitro assays. DPPH is an organic, nitrogen centred and stable free radical. It has absorption maximum at 517nm in alcoholic solution. The antioxidants having hydrogen donating ability convert purple colored DPPH radical into yellow colored non-radical diphenylpicrylhydrazine ,[7,21,22,23] In the present study, we evaluated radical scavenging activity of various parts of *G. montana* by DPPH assay. The result of radical scavenging activity of extracts from different parts of *G.montana* is shown in Figure 2. The extract displayed concentration dependent activity also increased. Among extracts, leaf extract (IC_{50} value 10.42µg/ml) exhibited stronger scavenging efficacy followed by flower (IC_{50} value 15.54µg/ml) and fruit extract (IC_{50} value 593.78µg/ml). Fruit extract showed negligible scavenging potential when compared to other extracts. At 100µg/ml concentration, scavenging effect of leaf, flower and fruit extracts was 94.73, 92.10 and 9.21% respectively. At concentration 6.25 and 12.5µg/ml concentration, fruit extract did not show scavenging effect. The scavenging effect of ascorbic acid (IC_{50} value 4.53µg/ml) was higher than that of extracts. Though the scavenging effect of extracts of *G. montana* were lesser than that of ascorbic acid, it is evident that the extracts showed hydrogen donating ability and therefore the extracts could serve as free radical scavengers,

acting possibly as primary antioxidants.[21] In similar studies, Bhavita *et al.* [16] and Gawade [24] showed the antioxidant effect of leaf and stem extracts of *G. montana* by various assays.

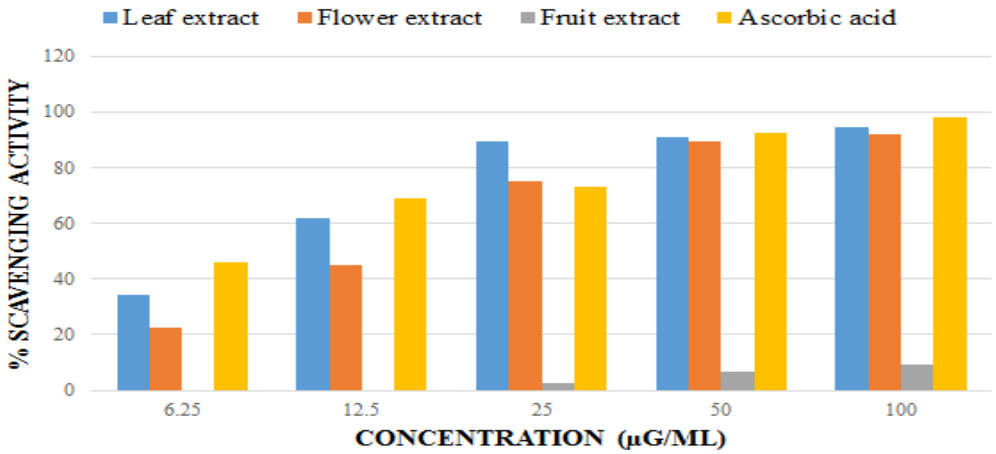

Figure 2: DPPH radical scavenging activity of extracts of *G. Montana.*

CONCLUSION

In the present *in vitro* study, the leaf, flower and fruit extract of *G. montana* exhibited antimicrobial and radical scavenging activity. The observed bioefficacies of plant could be ascribed to the presence of bioactive principles in the extracts. The plant, in suitable form, can be used to prevent and control/treat microbial infections and oxidative damage. Further, isolation of active components from extracts and their bioactivity determinations are to be conducted.

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