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ARTICLE

Studies on Phytochemicals, Minerals, Antioxidants and Efficacy of Crude Extracts of Therapeutic Herbal *Potentilla reptans* L. on Certain Pathogenic Causal Agents

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ABSTRACT

The crude extract of indigenous herbal *Potentilla reptans* L. habitat wild belonging to *Rosaceae* family, has been explored in search of therapeutic compounds that amalgamate as associative phytochemical and phyto mineral constituents. A number of novel bioactive organic compounds *viz*. antioxidant (30.8 µg/mL), saponin (30 mg/g), flavonoid (10.39 mg/g), alkaloid (30 mg/g), phenol (4.33 mg/g,) tannin (30.74 mg/g) and minerals like magnesium (4.55 mg/g), iron (1.12 mg/g), sulfur (1.85 mg/g), potassium (1.26 mg/g), manganese (0.10 mg/g), calcium (5.16 mg/g), phosphorous (0.14 mg/g), zinc (0.12 mg/g) and copper (0.06 mg/g), an indispensable sources of precious chemo-diversity, the only means for survivability of species forever on earth have depicted. In an antimicrobial test with crude extracts, a strong positive effect against *Staphylococcus aureus* in bacteria and *Candida albicans*, *Microsporum gypseum* in fungi by all extracts of MeOH, EtOAc and $CHCl_3$ was authenticated to the aged old traditional health care system of herbals and novel to unique in home remedy prescriptions.

KEYWORDS

Potentilla reptans, Bioactive constituents, Antimicrobial activity, Minerals.

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INTRODUCTION

It is well known proven fact that the phytochemicals have long been recognized to possess many properties including antioxidant, antiallergic, anti-inflammatory, antiviral anti-proliferative and anticarcinogenic [1]. *Potentilla reptans* is one among the high valued wild habitat herbal having significance medicinal prescription which is often practiced in Manipur, India. Further, the species *Potentilla reptans* (*Rosaceae*) is a cosmopolitan within the tropics and subtropics, with some species extending into the temperate regions [2,3].

The herbal plant is not only used as an anticoagulant, anti-septic, depurative and febrifuge but also employed in decoction or the fresh leaves by crushing and applying externally as poultice [4]. Further the herbal is employed in the treatment of skin disorders like boils and abscesses, weeping eczema, ring-worm, mouth complicacies *viz*. stomatitis, laryngitis, acute ton-sillitis, injuries of bites from snake and bug other traumatic injuries including swellings and activating blood circulation [4].

In recent years, a growing interest within the advancement of so-called functional foods has validated that they show

physiological benefits additionally to nutritional and energetic, for instance, antihypertensive, antioxidant or anti-inflammatory [5]. Among the various compounds with functional properties, antioxidants scored the foremost widely studied [6,7]. These compounds can play an immense and pivotal role in food technology because of their usefulness against lipid peroxidation. The important role of antioxidants in human health has been demonstrated, thus increasing the interest in such products and their demand by consumers [8]. Henceforth, the present work was designed to detect the bioactive compounds, antioxidants and minerals from the herbal plant *Potentilla reptans* and to investigate the antimicrobial activities of plant extract against certain human pathogenic fungi and bacteria.

EXPERIMENTAL

The plant, *Potentilla reptans* was collected from hillside Kakching Uyock Ching, Kakching district, India in August-September 2020. The plant materials were identified by the Botanical Survey of India (BSI), Shillong, India and the voucher specimen was deposited at Waikhom Mani Girls' College, Thoubal, India. The plant materials were cleaned, rinsed with deionized water and allowed to evaporate at room temperature and grind into uniform powdered with utmost care so as not to contaminate with dust. The coarse powder of the dried plant was extracted with different solvents *viz*. ethyl acetate, chloroform and methanol.

Determination of mineral elements: The major elements of calcium, phosphorous, potassium, magnesium and trace elements comprising iron, zinc, copper, manganese and sulfur were determined in the herbal plant according to the reported methods [9,10].

Determination of phytochemicals: Determination of free radical scavenging of plant extracts by the use of DPPH radical [11] and determination of alkaloid, saponin, total flavonoid content, total phenol and tannin were also determined according to the published methods [12,13].

Test microorganisms: The antimicrobial activities of different extracts of the medicinal plant *Potentilla reptans* include five bacteria *viz*. *Escherichia coli* (Gram-negative), *Pseudomonas putida* (Gram-negative), *Pseudomonas auriginosa* (Gram-negative), *Staphylococcus aureus* (Gram-positive), *Klebsiella pneumoniae* (Gram-negative) and five fungi *viz*. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans* and *Microsporum gypseum.*

Media: The Luria Bertani agars, Luria Bertani broth and potato dextrose agar (procured from Hi-media Laboratories, India), were appropriately used for the concerned microbes.

Antimicrobial agents: Amphotericin-B (75 µg in 2.5 mL of water) and chloramphenicol (90 µg in 3 mL of MeOH) were used as a control for bacteria and fungi, respectively.

Antimicrobial activity: Antimicrobial activity was accounted for using the modified paper disc method [14-17]. The paper disc was prepared by taking aliquots of 1 mL of each of the different extracts in separate eppendorf tubes. The sterilized paper disc prepared from the Whatman paper (dia. 5.42 mm) was dipped in the different extracts at the different concentrations for 1 h. After thoroughly soaked, the paper discs were incubated in the oven at 45 ºC overnight to evaporate the solvent from the paper disc. Approximately 13 mL of nutrient agar was poured into each sterilized petri-plate (90 mm) for base agar. Cell suspensions with the strength of 10^8 CFU/mL cells for bacteria and 10^7 CFU/mL cells for fungi were prepared. Each bacterium's 24 h broth culture and the fungus's 3-day culture were inoculated in previously melted and cooled soft agar (5 mL) at 45-50 ºC. After mixing well, the soft agar was poured over the base agar plate and after proper solidification of the soft agar, the paper disc previously prepared with its control was placed over the solidified agar plate. The bacterial plates were then incubated at 35 ºC for 24 h and the fungal plates at 25 ºC for 3 days.

Minimum inhibitory concentration (MIC): The determination of minimum inhibition concentration (MIC) was carried out by placing the paper discs in increasing or decreasing the concentration of the extract over the petri plate containing soft agar layered over the base agar plate [18].

Statistical analysis: The data represent the mean of replicates ± standard deviation (S.D.). Multiway analysis of variance was used to confirm the validity of the findings.

RESULTS AND DISCUSSION

Phytochemical constituents and its expository of *Potentilla reptans* yields a number of organic compounds inclusive of antioxidant and in different strengths saponin 30 mg/g, flavonoid 10.39 mg/g, alkaloid 30 mg/g, phenol 4.33 mg/g, tannin 30.74 mg/g and antioxidant ranged upto 30.8 µg/mL (Table-1). The mineral composition of the test herbal was observed with various concentrations *i.e.* calcium account 5.16 mg/g, in top followed by magnesium 4.55 mg/g, potassium 1.26 mg/g, phosphorous 0.14 mg/g, sulfur 1.85 mg/g, manganese 0.10 mg/g, iron 1.12 mg/g, zinc 0.12 mg/g, copper 0.06 mg/g and cobalt nil (Table-2).

Before isolating the active biochemical markers, it is essential to get a rough concept of the various classes of chemical constituents contained in the extract by estimating the concentrations of phytoconstituents in the plant material. The presence of these bioactive compounds has empathetically emphasized the medicinal potentials of the tested herbal. Similar results have been reported from different plants [8]. The antioxidant content in the *Potentilla reptans* herbal plant ranged upto 30.8 µg/mL under the free radical scavenging activity technique confirming the unique presence of the phytocompounds in *Potentilla reptans.*

TABLE-1
PHYTOCHEMICAL CONSTITUENTS OF FLAVONOID, SAPONIN, ALKALOID, PHENOL, TANNIN AND ANTIOXIDANT OF THE *Potentilla reptans*

Plant species	Antioxidant IC_{50} (µg/mL)	Flavonoids (mg/g)	Saponins (mg/g)	Alkaloids (mg/g)	Phenol (mg/g)	Tannin (mg/g)
Potentilla reptans	30.8 ± 0.35	10.39 ± 0.02	30 ± 0.57	30 ± 1.52	4.33 ± 0.25	30.74 ± 0.15

The values are mean of replicates with ± standard deviations.

TABLE-2
COMPOSITION OF MINERAL ELEMENTS OF *Potentilla reptans* (mg/g)

Plant parts	Mineral elements									
	K	Mg	P	Ca	S	Fe	Zn	Mn	Cu	Co
Whole plants	1.26±0.03	4.55±0.11	0.14±0.01	5.16±0.02	1.85±0.25	1.12±0.02	0.12±0.15	0.10±0.01	0.06±0.05	ND*

Values are the mean of replicates with ± standard deviation. *ND = Not detected.

The phenolic compounds content ranged upto 4.33 mg/g in *Potentilla reptans*, whereas the content of alkaloids ranged from 30 mg/g among the phytoorganic compounds. The flavonoids accounts upto 10.39 mg/g with other phytochemical compounds (Table-1), whereas the presence of saponin with 30 mg/g were also confirmed. And at last the tannins, which is believed to be the principal active substance of several plants utilized in folk medicines were found to be in the range of 30.74 mg/g. An analogous trend was reported earlier from other therapeutic plants [19].

Table-2 exhibit the mineral potential of K (12.6 mg/g), Mg (4.5 mg/g), P (0.19), Ca (5.16 mg/g), Fe (1.1 mg/g), Zn (0.13 mg/g), Mn (0.1 mg/g), Cu (0.06 mg/g), S (1.08 mg/g) of test herbal *Potentilla reptans* demonstrating the content of responsive pooled minerals which are active functionaries of living system in a living body. Minerals help to maintain a normal water balance within the living body, stimulate a healthy immune system (iron), fight cancer cells (manganese), antibiotic capabilities (sulfur), help wounds heal, production of white blood cells (zinc), *etc.* [18].

Antimicrobial activity: The antimicrobial activity of the crude extract and solvent fractions of *Potentilla reptans* for whole aerial parts *viz*. MeOH, EtOAc and $CHCl_3$ was assayed with the bacterial types of *Escherichia coli, Klebseala pneumonea, Pseudomonas auriginosa, Pseudomonas putida*, *Staphylococcus aureus* and fungal types of *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Microsporum gypseum, Aspergillus niger*.

The antimicrobial activity of crude extract and solvent fractions of the whole aerial parts *Potentilla reptans* had a broad spectrum of activity against both the Gram-positive and Gram-negative bacterial isolates. However, it was observed that the activity was different for different fractions. Among them EtOAc accord the most active extract against *Pseudomonas putida* (15 mm) *Staphylococcus aureus*, *Klebseila pneumonia* in bacteria, *Candida albicans, Microsporum gypseum* (10 mm), followed by MeOH extract active against *Pseudomonas putida, Staphylococcus aureus* in bacteria and *Candida albicans* and *Microsporum gypseum* (15 mm) in fungi; then $CHCl_3$ extract which active against *Staphylococcus aureus* only in bacteria and *Candida albicans* (13 mm) and *Microsporum gypseum* (15 mm) in fungi.

The results clearly showed that the ethyl acetate extract of *Potentilla reptans* had potent antibacterial activity against *Pseudomonas putida* and *Staphylococcus aureus* but had a much weaker effect against *Pseudomonas aeruginosa*. These results add more evidences to the hypothesis that methanolic extract of *Potentilla reptans* is selective in its antibacterial activity, as it solely inhibits the growth of *Pseudomonas putida* and *Staphylococcus aureus* (Table-3). In case of chloroform extract of *Potentilla reptans*, the antibacterial activity against *Staphylococcus aureus* is only effective.

Table-4 revealed the methanolic extract of *Potentilla reptans* with very low suppression of MIZ, 10 mm and 15 mm on *Candida albicans* and *Microsporum gypseum* as against the 30 mm and 20 mm control. Chloroform extract of *Potentilla reptans* on *Candida albicans* and *Microsporum gypseum* with low suppression of 13 mm and 15 mm against 30 mm and 20 mm, respectively in control. With ethyl acetate extract, *Potentilla reptans* the test fungi react low suppression on *Candida*

TABLE-3
ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACT OF THREE DIFFERENT SOLVENT FRACTIONS

Organism	Bacteria zone of inhibition (mm)			
	MeOH extract	$CHCl_3$ extract	EtOAc extract	Control
E. coli.	–	–	–	14 ± 0.57
K. pneumonea	–	–	–	14 ± 1.52
P. auriginosa	–	–	10 ± 0.57	14 ± 1.52
P. putida	15 ± 1.00	_	15 ± 1.52	13 ± 0.57
S. aureus	18 ± 0.57	15 ± 1	15 ± 0.57	14 ± 1.00

Values are mean of replication with ± standard deviation (–) no zone inhibition was found.

TABLE-4
ANTIFUNGAL ACTIVITY OF CRUDE EXTRACT OF THREE DIFFERENT SOLVENT FRACTIONS

Organism	Fungus zone of inhibition (mm)			
	MeOH extract	$CHCl_3$ extract	EtOAc extract	Control
Aspergillus flavus	–	–	–	25 ± 0.57
Aspergillus fumigatus	–	–	–	30 ± 1.00
Aspergillus niger	–	–	–	20 ± 0.57
Candida albicans	10 ± 0.57	13 ± 1	15 ± 0.57	30 ± 0.57
Microsporum gypseum	16 ± 1.52	15 ± 1	10 ± 0.57	20 ± 1.15

Values are mean of replication with ± standard deviation (–) no zone inhibition was found.

albicans and *Microsporum gypseum* which accord 15 mm and 10 mm against 30 mm and 20 mm of control.

The impact of antifungal activity of MeOH, $CHCl_3$ and EtOAc extract of *Potentilla reptans* on *Candida albicans* and *Microsporum gypseum* with low supression of fungal growth even lower than control. The current investigation indicates that the MeOH, $CHCl_3$ and EtOAc extracts of *Potentilla reptans* contains bioactive components but no significant impact on *Aspergillus flavus*, *A fumigatus* and *A. niger*. These findings highlighted that extracts of *Potentilla reptans* are not effective to use in controlling all the test fungi. These findings were concordance with that of the different workers in different plant extracts at different places [20,21].

The present finding vividly showed that *Potentilla reptans* has selective antibacterial and antifungal activities hence it has full potential to use in the treatment of those ailments caused by those microorganisms.

Conclusion

Present study provides clear evidence that the *Potentilla reptans*, due to its high levels of phytochemicals and minerals, has unique therapeutic potential and can be used to cure a wide range of serious illnesses. Further, the potent bioactive compounds in the extract of *Potentilla reptans* in pharmacological pharmacokinetics and pharmacodynamics in the elimination of bacterial microbes enact selectively and thereof required to adjustment in the management of diseases with associated microbes. Phytochemically, the majority of the phytoconstituents present in *Potentilla reptans* are polyphenolics components with phenolics and tannins as the most dominant phytochemicals.

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