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Phytochemical Analysis and Evaluation of Leaf and Root Parts of the Medicinal Plants and Herbs of Banswara, Rajasthan

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ABSTRACT

A floristic and ethno botanical survey of medicinal plants was conducted in the near mahi river of District Banswara. About 78 percent of the population, largely tribes, still likes to utilise herbs for various diagnostic purposes, however the rural community employed traditional treatments in conjunction with modern medicines. Tribal cultures are well-versed in ethno botanical knowledge and the usage of medicinal plants for the treatment of various diseases, wounds, fractures, and other afflictions. In this study, 31 plant species were identified as being used by rural and tribal populations for various diagnostic purposes. The medicinal properties of these plants were mostly gathered and based on folk practitioners through in-depth interviews with local physicians practicing indigenous medicine, village headmen, priests, and tribal people. The study reveals that diverse components of the plant, such as leaves, stems, bark, and roots, are utilised either directly or in various traditional formulas.

KEYWORDS: *Phytochemical analysis, leaf and root, medicinal plants, herbs*

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1. INTRODUCTION

Any atom or molecule with unpaired electrons is referred to as a free radical. Free radicals derived from oxygen, such as superoxide anion (O2), hydroxyl (OH•), hydroperoxyl (OOH), peroxyl (ROO), and alkoxyl (RO•) radicals, and non-free radicals, such as hydrogen peroxide (H2O2), hypochorous acid (HOCl), ozone (O3), and singlet oxygen (O3), are examples of reactive oxygen species (O2).Exogenously (respiration, peroxisome stimulation of polymorphonuclear leucocytes and macrophages) and endogenously (respiration, peroxisome stimulation of polymorphonuclear leucocytes and macrophages) it can be generated in live organisms (ionizing radiation, tobacco smoke, pollutants, pesticides and organic solvents). These free radicals are produced by our bodies to help them function properly, but an excessive amount can harm cells and tissues. In humans, it can cause oxidative damage to proteins, lipids, and DNA, as well as chronic disorders like cancer, diabetes, ageing, and other degenerative diseases.

Any chemical that delays or prevents oxidative damage to a target molecule is considered an antioxidant. Antioxidants are known for their capacity to scavenge free radicals due to their redox hydrogen donors and singlet oxygen quencher. Natural (plants) and synthetic (butylated hydroxyl toluene, butylated hydroxyl anisol, and tetra butyl hydro quinone) antioxidants can scavenge free radicals. However, synthetic antioxidants are being phased out in favour of natural antioxidants, which are thought to be safer and have fewer adverse effects. Many researchers have been interested in medicinal plants in recent decades to evaluate antioxidant phytochemicals such as phenols, flavonoids, and tannins, which have gotten increased attention for their possible involvement in disease prevention.

Hypochaeris radicata (H. radicata) is an edible perennial herb found in the hills near the Mahi river, at a height of 1500 metres above sea level. Anti-inflammatory, anticancer, antioxidant, antibacterial, antifungal, and antidiuretic effects are claimed to be present throughout the plant. In the traditional medicinal practise of Banswara, India, it is used to treat jaundice, rheumatism, dyspepsia, constipation, hypoglycemia, and renal disorders. However, there hasn't been much scientific support for this species' medical properties. To fill this gap, the current study used several alcoholic (petroleum ether, chloroform, ethyl acetate, and methanol) and aqueous extracts to conduct qualitative and quantitative phytochemical analyses and in vitro antioxidant activities of H. radicata leaf and root sections.

2. Literature Review

MUKESH KUMAR SHARMA (2016) The potential of natural vegetation, specifically Adhatoda vasica, is discussed in this research. Shekhawati region, generally known as historical heritage, geographical, and cultural zone in Rajasthan state,

India, is the subject of the investigation. Aside from that, this zone contains a unique combination of magnificent ecosystems, including riverine, sandy plain, sand dunes, and stony and rocky ecosystems. The sand dunes are the heart of Churu's environment. The Shekhawati zone contains a wide range of temperatures (semi-arid and arid), biotic and edafic conditions, physiography, and natural vegetation diversity, all of which have an impact on a wide range of natural ecosystems.

B B.B.S. KAPOOR AND SUNITA SWAMI (2016) Rajasthan's desert terrain is abundant in ethnomedicinal plants. These plants have long been used by tribal groups in this region for herbal and folk treatments. There is an urgent need to raise public understanding about the medicinal and economic importance of these plants, particularly among farmers, so that their heritage can be effectively used and exploited while also being saved and perpetuated for future generations through prudent management. This would ensure the preservation and protection of ethnomedicinal plant germplasm.

GITIKA AND MANOJ KUMAR (2016) During the seasons of 2015-2016, the ethno botany and traditional medicinal uses of several medicinal plants in Haryana were investigated. The ethno medicinal value highlighted by local informants led to the identification of 66 plant species belonging to 30 groups during the survey. Botanical names, plant families, local names, Hindi names, habits, plant parts used, and their medical purposes are all provided for each plant species. Moraceae, with six species, is the most dominant family in the current study.

NEHA MISHRA AND ARVIND PAREEK (2015) The goal of this study was to document the variety of Angiosperm species in India's Kota area. It also emphasises the medicinal qualities of plants found in this region. A total of 133 species and intraspecific taxa of plants were recorded during the floristic study, representing 99 genera and 46 families. The majority of the species have therapeutic value and are used to treat a variety of ailments including bronchitis, ulcers, diarrhoea, dysentery, wounds, eruptions, bone fractures, kidney stones, and respiratory, digestive, urinary, and skin issues. This initiative has the potential to expand knowledge of local plants and give financial support for future opportunities to document Rajasthan's flora.

3. Materials and methods

3.1. Chemicals

All of the substances used in this investigation were bought from HI-MEDIA Pvt. Ltd. in Bombay. The chemicals utilised were of the highest quality.

3.2. Collection and identification of plant materials

The plant H. radicata was found in the near hilly area of Mahi river region Banswara. This species' fresh leaf and root parts were washed under running tap water, dried in the shade at room temperature, andpulverised.

3.3. Extract preparation

To extract the polar and non-polar chemicals, powdered plant samples (50 g/250 mL) were extracted sequentially with petroleum ether, chloroform, ethyl acetate, methanol,and water using a Soxhlet apparatus at 55-85 ° C for 8-10 hours. The powdered pack material was air dried and then used for each solvent extraction. The different extracts' solvents were decreased at room temperature and kept at 4 ° C for further use. The dried plant extracts were then redissolved in dimethyl sulfoxide to obtain a 10 mg/10 mL solution for each extract, which was then testedfor antioxidant activity in vitro.

3.4. Preliminary qualitative phytochemical analysis

The secondary metabolites found in the various alcoholic and aqueous extracts of H. radicata leaf androot sections were identified using a preliminary qualitative phytochemical analysis.

3.5. Quantitative estimation of chemical constituency

3.5.1. Determination ofalkaloids

In a separate 250mL beaker, 200mL of 20% acetic acid was added to 5g of leaf and root powders, which were covered and left to stand for 4 hours. The volume of this solution-containing combination was lowered to one-quarter using a water bath. Concentrated ammonium hydroxide was applied to this sample drop by drop until the precipitation was complete. The entire solution was allowed to settle before the precipitate was collected and weighed using filtration. The following formula was used to determine the percentage of total alkaloid content:

Percentage of total alkaloids (%)=Weight of residue× 100/Weight of sample taken

3.5.2. Total phenolics content

Using the Folin-Ciocalteau reagent and the Sidduraju and Becker method, the total phenolics content of H. radicata was calculated. Separately, 20 g of leaf and root extracts were collected and diluted to 1 mL with distilled water. After that, 500 mL of diluted Folins- phenol reagent (1:1 with water) and 2.5 mL sodium carbonate Na2CO3 (20%) were added. The liquid was thoroughly mixed before being incubated in the dark for 40 minutes to develop colour. The absorbance was measured at 725 nm after incubation. Gallic acid was used to create a calibration curve, and linearity was found in the range of 10-50 g/mL. Using the standard curve, the total phenolics content in the plant extracts was quantified as mg of gallic acid equivalent (mg GAE/g extract).

3.5.3. Total flavonoids content

Zhishen et al. established a procedure for estimating total flavonoids content. A total of 1 mL of plant extracts were diluted in 200 litres of distilled water before being added to 150 litres of sodium nitrite (5%) solution. After 5 minutes of incubation, 150 L of aluminium chloride (10%) solution was added and allowed to stand for 6 minutes. Then 2 mL of sodium hydroxide (4 percent) solution was added and distilled water was added to make up to 5 mL. The liquid was thoroughly mixed before being left at room temperature for 15 minutes. At 510 nm, the absorbance was measured. The presence of flavonoids was indicated by the pink colour. Using the standard curve, the total flavonoids content was represented as rutin equivalent mg RE/g extract on a dry weight basis.

3.5.4. Estimation of tannins content

The Tannin content of H. radicata was calculated using the Siddhuraj and Manian technique. A total of 500 litres of extract were separated into test tubes and treated with 100 mg polyvinyl polypyrrolidone and 500 litres of distilled water. This solution was incubated for 4 hours at 4 degrees Celsius. The sample was then centrifuged for 5 minutes at 5 000 r/min, and 20 l of the supernatant was extracted. There are no tannins in this supernatant, simply basic phenolics (the tannins would have been precipitated along with the polyvinyl polypyrrolidone). The concentration of free

phenolics in the supernatant was measured at 725 nm and represented as a percentage of dry matter. The tannin content of the extract was determined based onthe above results:

Tannins (mg GAE/g extract) =Total phenolics (mg GAE/g extract)-Free phenolics (mg GAE/g extract)

3.5.5. Estimation of total saponins content

The method published by Makkar et al., which is based on the vanillin-sulphuric acid colorimetric reaction with certain modifications, was used to estimate total saponins concentration. 250 litres of distilled water were mixed with 50 litres of plant extract. About 250 litres of vanillin reagent (800 mg vanillin in 10 mL 99.5 percent ethanol) were added to this. After that, 2.5 mL of 72 percent sulphuric acid was added and thoroughly mixed. This solution was maintained at 60° C for 10 minutes in a water bath. It was chilled in ice cold water for 10 minutes before the absorbance was measured at 544 nm. The results were calculated usinga standard curve and represented as diosgenin equivalents (mg DE/gextract).

3.5.6. Ascorbic acid (vitamin C)

Klein and Perry's method for determining ascorbic acid was used. With 10 mL of 1 percent metaphosphoric acid, about 10 mg of dried plant powder were re-extracted. They were allowed to sit at room temperature for 45 minutes before being filtered through Whatman No. 4 filter paper. After mixing 1 mL of filter with 9 mL of 50 mol/L 2,6-dichloroindophenol sodium salt hydrate, the absorbance was measured at 515 nm for 30 minutes. The ascorbic acid concentration was determined using an authentic L-ascorbic acid calibration curve, and the results were represented in mg of ascorbic acid equivalent (mg AE/g extract).

3.6. In vitro antioxidant activities

3.6.1. DPPH radical scavenging activity

Blois' method was used to examine the potential of H. radicata extracts to scavenge DPPH• radicals, with some changes. A 0.2 mmol/L DPPH• solution in methanol was produced, and 500 mL of this solution was added to extract concentrations ranging from 50 to 250 g/mL. The mixture was vigorously shook before being set aside for 30 minutes at room temperature. The control was made in the same way as the sample extracts, but without the sample extracts, and the baseline correction was done with methanol. The absorbance of the plant samples was then measured using a spectrophotometer at 517 nm. The higher the radical scavenging activity, the lower the absorbance value. The antioxidants rutin, quercetin, butylated hydroxylanisole (BHA), and butylated hydroxytoluene (BHT) were compared to the results. The following formula was used to calculate the ability of DPPH• radical scavenging activity:

$$DPPH^{\bullet} \text{ scavenging effect (\% of inhibition)} = (A_0 - A_1) \times 100/A_0$$

The absorbance of the control is A0, and the absorbance of the sample extracts is A1. Using linear regression analysis, the IC50 value (the microgram of extract required to scavenge 50% of the radicals) was obtained. Antioxidant activity is shown by alower IC50value.

3.6.2. Nitric oxide radical scavenging activity

Sreejayan and Rao's approach was used to determine nitric oxide radical scavenging activity. The Griess reagent was used to quantify nitric oxide radicals generated by sodium nitroprus side solution. In an aqueous solution of sodium nitroprusside at physiological pH, nitric oxide radicals form spontaneously, interfering with oxygen to produce nitrite ions. Nitric oxide scavengers compete with oxygen, reducing the formation of nitrite ions. Various amounts of the extracts (250-450 g/mL) were added to 3 mL of sodium nitro prusside (10 mmol/L) in phosphate buffer saline (0.2 mmol/L, pH 7.4) and incubated at 25 ° C for 150 minutes. The Griess reagent (1% sulphailamide, 2% orthophosphoric acide, 0.1 percent N-1-napthylethylenediamine dihydrochloride) was then added to the mixture. The percentage of inhibition was determined using the same procedure as DPPH• and the absorbance readings were measured at 546 nm. High nitric oxide radical scavenging activity is demonstrated by the lower in goptical density readings. The IC50 value was computed and compared to the antioxidants rut in, quercetin, BHA, and BHT, as well as other common antioxidants.

3.6.3. Reducing power activity

Yildrim et al approach's was used to determine H. radicata's reducing ability. Various amounts of plant extracts (300-700 g/mL) were combined with 1 mL of freshly made 1 percent potassium ferric cyanide and 1 mL of 0.2 mol/L sodium phosphate buffer (pH 6.6). For 20 minutes, the mixture was incubated in a water bath at 50° C. Then 1 mL of 10% trichloroacetic acid was added and centrifuged for 10 minutes at 3000 r/min. The supernatant (2 mL) was combined with 2 mL distilled water and 500 mL ferric chloride (1 percent) (freshly prepared). At 700 nm, the absorbance was measured. Greater reducing power is shown by a higher absorbance of the reaction mixture. The results were compared to those of rutin, quercetin, BHA, and BHT, which are common antioxidants.

RESULTS

The phytochemical screening of plant components revealed that tannins, terpenoids, cardiac glycosides, and flavonoids were abundant in leaves, stems, roots, and in vitro callus and regenerated leaves (Table 1). In comparison to leaves and roots extracts, seeds had the most activity, whereas aqueous extracts of in vitro generated callus and leaves had little action against bacterial strains (Table2,3).None of the aqueous extracts, on the other hand, demonstrated any action against M. luteus. Ethanolic extracts of leaves, roots, in vitro developed callus, and regenerated leaves all exhibited significant activity against all strains, but ethanolic extract of seeds showed the most activity against all strains. Using the well diffusion method, it was discovered that ethanolic extracts of seeds had the highest antibacterial activity against E. coli, followed by B. cereus>B. subtilis>M. luteus. The maximum activity of ethanolic seed extract against E. coliwas 15.4 mm, the zone of inhibition against B. cereus was 13.2 mm, the zone of inhibition against B. subtilis was 11.13 mm, and the zone of inhibition against M. luteus was 8.9 mm (Table 2).

Table 1: Qualitative analysis of the phytochemicals of H. radicata L. seeds, leaves, roots, callus, in vitro regenerated leaves.

Metabolites	*In vivo*	Callus	*In vitro* leaves
Tannins	+	+	+
Terpenoid	+	+	+
Saponins	+	+	-
Steroid	+	+	+
Flavonoid	+	+	+
Phlobatannin	-	-	-
Cardiac- Glycoside	+	+	+
Anthraquinone	-	-	-

+: Present, - : Absent

Table 2: Antibacterial activity of H. radicata L. aqueous and ethanolic extracts against bacterial strains by well diffusion method.

Plant samples	*E. coli*	*B. cereus*	*B. subtilis*	*M. luteus*
In vivo				
Aqueous seeds	3.80±0.04	3.70±0.00	1.20±0.04	-
Ethanolic seeds	15.40±0.08	13.20±0.08	11.13±0.04	8.90±0.09
Aqueous leaves	-	-	-	-
Ethanolic leaves	8.10±0.08	8.03±0.04	7.53±0.04	5.13±0.04
Aqueous roots	-	-	-	-
Ethanolic roots	3.40±0.04	4.43±0.04	1.60±0.04	1.20±0.04
In vitro				
Aqueous callus	-	-	-	-
Ethanolic callus	3.16±0.04	3.33±0.04	2.06±0.04	-
Aqueous leaves	-	-	-	-
Ethanolic leaves	2.26±0.04	2.43±0.04	2.10±0.00	1.10±0.00
Streptomycin	20.20±0.80	25.10±0.50	19.20±0.20	18.50±0.04

Table 3: Antibacterial activity of H. radicata L. aqueous and ethanolic extracts against bacterial strains by disc method.

Plant samples	*E. coli*	*B. cereus*	*B. subtilis*	*M. luteus*
In vivo				
Aqueous seeds	3.00±0.000	2.50±0.00	1.20±0.00	-
Ethanolic seeds	11.66±0.470	13.16±0.12	8.80±0.12	6.80±0.21
Aqueous leaves	1.86±0.040	1.16±0.47	-	-
Ethanolic leaves	8.83±0.040	8.53±0.23	6.06±0.09	3.06±0.75
Aqueous roots	2.40±0.081	1.16±0.07	1.86±0.04	-
Ethanolic roots	3.70±0.047	4.13±0.39	2.70±0.08	-
In vitro				
Aqueous callus	-	-	-	-
Ethanolic callus	3.46±0.000	3.40±0.74	2.10±0.08	-
Aqueous leaves	-	-	-	-
Ethanolic leaves	3.33±0.040	3.32±0.65	3.06±0.70	1.23±0.68
Streptomycin	22.36±0.030	25.95±0.37	20.08±0.06	19.31±0.62

Among the other bacteria, Micrococcus lute us demonstrated the least amount of inhibition. Similar results were obtained using the agar disc approach. In vivo, ethanolic seeds (11.6 mm) had the highest efficacy against E. coli, followed by ethanolic leaves (8.83 mm). The ethanolic extract of seeds had the highest effectiveness against B. cereus, with an inhibition zone of 13.16 m. Similarly, ethanolic seeds had the highest effectiveness against B. subtilis (8.86 mm). Only ethanolic extracts of seeds and leaves demonstrated action against M. luteus, with inhibition zones of 6.8 and 3.2 mm obtained later. With the agar disc method, the trend was B. cereus >E. coli > B. subtilis >M. luteus (Table 3). Antibacterial activity of ethanolic extracts of roots, calluses, and in vitro regenerated leaves was also very low against the strains. It's probable that because of the ideal in vitro environmental conditions, calluses and regenerated leaves acquire fewer active components than in vivo plant parts, and so have lower activity.

Conclusion

Traditional healers generally utilise water as a solvent, however we discovered in our investigation that ethanol-based extracts had more consistent antibacterial efficacy than water-based extracts. This could be due to the active ingredients' insolubility in aqueous solutions, but ethanol extract demonstrated some antibacterial activity, as Parekh et al. discovered in methanol extract of various medicinal plants (2005). The impact of solvent composition on extract efficacy will be investigated further in experiments utilising

solvents of varied polarity (Romero et al., 2005). Similarly, antibacterial and antifungal activities of H. radicata acetone extract and seed oil have been reportedagainst Staphylococcus aureus, Bacillus cereus, Salmonella typhii, Pseudomonas aeruginosa, and Bacillus subtilis, as well as antifungal activities against Aspergillus niger, Fusarium moniliforme, and Penicilium citrinum (Singh et al., 2005). Decoctions or infusions of H. radicata produced in water have been used to heal diseases for many years. Aqueous extracts of H. radicata were found to have broad antibacterial activity against Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Salmonella typhimurim, Shigella flexneri, and Salmonella typhii (Arora and Kaur, 2007). According to the results of antibacterial activity against all strains, ethanolic seed extract and aqueous extract have the highest activities, indicating that seeds have higher potential for microbial activity than other plant parts. It could be due to the ingredients found in H. radicata seeds,which contain high levels of carvone (55.2%), limonene (16.6%), dill-apiole (43.2%), linoleic acid (23.1%), and anethole (11%). (Singh et al., 2005). The greater activity of extracts canbe explained by the chemical structure of its primary ingredients, such as dill-apiole and anethole, which possess an aromatic nucleus with a polar functional group that is known to establish hydrogen bonds with the target enzyme's active sites (Farag et al., 1989). It has been observed that its seed oil has low antibacterial action against gram-negative bacteria, which could be due to changes in composition according to variety; agronomicpractise and processing, which can also affect concentrations of active components, affecting antimicrobial characteristics (Delaquis et al.,2002).

In conclusion, the plant's seeds have great antimicrobial potential, but other plant elements such as leaves, roots, callus, and in vitro regenerated leaves all have the abilityto display antimicrobial activity, according to the findings. This plant can be subjected to further enhancement and isolation of medicinal antimicrobials, as well as pharmacological testing.

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