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PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF NOVEL HERBAL FORMULATIONS

Rahul Mahamuni
Department of Environmental Science, S.B.E.S. College of Science, Aurangabad, Maharashtra.
Email: rahulrmahamuni@gmail.com
P.S.Geetha Malini
Department of Chemistry, Quaid-E-Millath Govt., College for Women, Chennai, Tamil Nadu.
Email: gita1krsna@gmail.com

Selvamohan. T
Department of zoology, Rani anna government college, Tirunelveli, Tamil Nadu.
Email: tsmohan71@gmail.com

M. I. Niyas Ahamed
Department of Biochemistry, Sacred Heart College (Autonomous), Tirupattur, Tamil Nadu.
Email: driniyasahamed@shctpt.edu

C. Kiruba Rani
Department of Biochemistry, Vellalar College for Women (Autonomous), Erode, Tamil Nadu.
Email: kripsbio@gmail.com

Niti Chawla
Department of Biotechnology, Chaudhary Bansi Lal University, Bhiwani, Haryana.
Email: nitichawlahans@gmail.com
***Author for correspondence: driniyasahamed@shctpt.edu**

ABSTRACT

This research set out to determine whether or not the crude methanolic extract of *A. santolinifolia* Turcz. Ex Besser possessed any phytochemical, antioxidant, or antibacterial properties. The antioxidant activity was measured with 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzthiazoline-6- sulphonic acid (ABTS) assays, with the methanolic extract of *Artemisia santolinifolia* root showing the highest scavenging activity (DPPH) at 61.31 g/ml and the leaves showing the lowest at 51. Similarly, *A. santolinifolia* roots showed the highest activity in (ABTS) (89.16 g/ml), whereas leaves showed the lowest activity (68.14 g/ml). At doses as low as 25 g/ml, 50 g/ml, and 100 g/ml, crude methanolic extracts demonstrated substantial suppression of all tested microorganisms. *A. santolinifolia* AsL leaf extract demonstrated MIC values of 12.5 g/ml against gram-positive bacteria B. subtilis, 50 g/ml against gram-positive bacteria S. aureus, and 37.5 g/ml against gram-negative bacteria P.

aeruginosa, which are all very close to the response of conventional ciprofloxacin. Based on the results of the current study, we recommend using *Artemisia santolinifolia* root (AsR) for the treatment and management of various infectious diseases because of its high antioxidant capacity and *Artemisia santolinifolia* leaf (AsL) because of its good antibacterial activity.

Keywords: Antioxidant Potential, Antibacterial Activity, Phytochemicals, *Artemisia santolinifolia*.

INTRODUCTION

Animal and plant health can be profoundly affected by medicinal herbs. According to the research, pharmaceutical firms rely heavily on medicinal herbs. Narayanaswamy and Balakrishn (2011), Balakrishn et al. (2013), Kotan et al. (2013), and others have established that the majority of the phytonutrients found in medicinal plants possess antioxidant, antibacterial, anti-inflammatory, phytotoxic, and cytotoxic effects. Plants used in medicine are particularly rich in therapeutic compounds, making them a prime source for crude drugs. Traditional medicine provides the best value in healthcare for the general public [1-9].

Natural products with innovative medicinal characteristics are sold, and there is widespread use of herbal medications for healthcare. The low toxicity and diverse pharmacological applications of plants have prompted researchers all around the world to study their medicinal characteristics. The usage of several types of medicinal herbs in the disorder types. Traditional medicine has been using plant extracts in a wide variety of medicinal preparations to treat a wide variety of disorders for thousands of years. Many essential oils and secondary metabolites make Artemisia, one of the many varied genera in the family Asteraceae, an important part of modern medicine. Artemisia species are being divided into distinct taxonomic classes according to their biological traits. Plants that have been extracted for their phenolic compounds might improve the overall quality of food by inhibiting the growth of microorganisms and the oxidation of lipids.

The presence of antibacterial and anti-oxidative substances in the tissues of many plant species and herbs gives them a preservation function. Neurodegenerative disease, atherosclerosis, aging, and cancer are only few of the disorders for which an adverse association between antioxidant level and incidence is known. The purpose of this work is the evaluation of antibacterial effect for *A. santolinifolia* against gram positive and gram-negative bacteria to standard antibiotics e.g., ampicillin and ciprofloxacin at varied concentration [10-16].

MATERIAL AND METHODS

The research study was carried out in the Department of Environmental Science, S.B.E.S. College of Science, Aurangabad, Maharashtra.

COLLECTION AND AUTHENTICATION

The specimen was collected, identified, and then shade dried before being weighed. The specimens were powdered after drying and then submerged in methanol for a set amount of time while being gently shaken every so often. A semisolid mass was obtained after the material was filtered and concentrated using a rotary evaporator.

PHYTOCHEMICAL ANALYSIS

All plant species were analyzed, down to the cellular level, employing a battery of standard chemical assays for the presence of bioactive chemicals.

Tannin analysis: The following procedure, developed by DOSS (2009) [5], was used to quantify the presence of tannins across different fractions. Twenty milliliters of distilled water were heated with 50 milligrams of each component in a test tube, then filtered. A few drops of 0.1% ferric chloride were added to each test tube, and the resulting color change was studied; the presence of tannins was indicated by a brownish green or blue-black hue.

To determine whether or not a plant extract contains phenols, it can be tested by adding a few drops of ferric chloride solution and seeing the resulting bluish-black color, as described by Silva et al. (2017) [20].

Saponins were tested for, with the normal technique for saponins being carried out in different fractions according to Mir et al. (2013) [12]. Each sample was cooked for five minutes in a water bath, yielding 20 mg of material, which was then filtered. For froth production, 10 ml of each filtrate was combined with 5 ml of distilled water and shaken briskly. After adding three drops of olive oil to the foam and shaking it hard, an emulsion formed.

According to Mir et al. (2013) [12], terpenoids were detected in different percentages of the sample. Each extract was diluted to 5 ml (1 mg/ml) in chloroform, and then 3 ml of concentrated H_2SO_4 was added to create a layer. The presence of terpenoids revealed by reddish brown coloring of the interface.

To test for flavonoids, follow the standard methodology established by Prabhavathi *et al.* (2016) [26] combine 25 mg of extract with 50 ml of cold water, mix rapidly, and then add 2 to 3 ml of NaOH solution. If a yellow color develops, flavonoids are present.

To conduct a protein assay following the protocol established by Silva et al. (2017), [20] 5mg extract should be diluted in 5ml water.

The addition of Million's reagent causes the surface to turn a hazy ash color, revealing any proteins present.

Five milligrams of extract were combined with five milliliters of chloroform and a few drops of hydrogen peroxide for a steroid test, as per the standard protocol outlined by Islam *et al.* (2016) [6]. A steroid was present if a reddish-brown color formed.

For the carbohydrate test, 5 mg extract was dissolved in 10 ml of water, and the sample was taken at 2 ml. According to Prabhavathi *et al*. (2016) [26], a purple tint resulted from mixing 5 ml H2SO4 with a few drops of Molisch's reagent.

ANTIOXIDANT ACTIVITY

Plant extracts were tested for their antioxidant activity using DPPH and ABTS free radicals. The antioxidant activity of the root, flower, leaf, and stem was tested using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. DPPH was dissolved in methanol at a concentration of 0.004% (w/v), and then 50 L of a 2.0 mg/mL leaf extract (or 80% methanol as a blank) was added to the mixture. It was incubated for 30 minutes in a dark box. The same method was performed on plant parts such as leaves (2.0 mg/mL), roots (2.0 mg/mL), and stems (2.0 mg/mL). The gold standard was ascorbic acid. The absorbance at 517 nm was measured after a 30-minute incubation period. To determine the DPPH scavenging activity (in percent), we

used the following equation.
Scavenging activity against DPPH radicals (in %) = [(A0 A1) / A0] 100

In which the absorbance of the plant sample is denoted by A1, and the absorbance of the control is denoted by A0. Oktay et al. (2003) [13] tested the scavenging percentage of several plant extracts with that of positive controls. The 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical cation decolorization assay was used to measure the antioxidant capacity of plant materials. Water containing ABTScation radical was created by reacting 7 mM ABTS with 2.45 mM potassium persulfate, and was stored in the dark at room temperature before use. To achieve an absorbance of 0.700 at 734 nm, an ABTS+ solution was diluted with methanol. The absorbance was measured 30 minutes after adding 5 l of plant extract to 3.995 ml of diluted ABTS+ solution and mixing thoroughly. A solvent blank was included in every test. There were five separate sets of measurements taken. Percentage inhibition of absorbance at 734 nm was obtained using the following formula:
The scavenging efficiency of ABTS+ (in percentage) = ((AB-AA)/ AB) 100 (2)
The absorbance of the ABTS radical in methanol is AB, and the absorbance of the ABTS radical in the sample extract or standard is AA (Rajurkar and Hande, 2011) [14].

Agar well diffusion testing of approximately 20 ml in sterile Mueller-Hinton petri plates was used to screen AsL, AsR, and AsS from various sections of selected species for antibacterial activity against both gram-positive and gram-negative bacterial strains. It was decided to pour some agar and let it set up. A sterile cotton swab was dipped in a bacterial culture (106 to 108CFU/ml), and the agar plates were equally inoculated by swabbing before the wells were formed using a sterilized cork-borer (6mm in diameter). Each pre-marked well received 100 l of a flavone's derivative solution of varying concentrations, which was then chilled for 30 minutes to facilitate diffusion. The plates were kept in a 37oC incubator for a whole day. The well-outside average zone of inhibition was measured from three separate plates for each treatment. DMSO (dimethyl sulfonic acid) served as a negative control. The antibacterial potential was measured in terms of the zone of inhibition (mm) and compared to that of two commonly used antibiotics, ampicillin and ciprofloxacin.

MINIMUM INHIBITORY CONCENTRATION (MIC)

Concentrated extracts of carefully chosen plant and animal species, the minimum inhibitory concentration (MIC) for suppressing the growth of any of the aforementioned microorganisms was retested. To find MICs, a broth dilution method was used. To get the desired concentration range, a stock solution of each component was prepared in dimethyl sulfoxide (DMSO), and then diluted serially. Each sterile test tube with a known concentration of test substance had a uniform volume of nutritional broth medium added to it. The inoculum, which was a broth culture of microorganisms grown overnight, was added to each tube. After 24 hours in a 37oC incubator, the turbidity was checked in the tubes. In a control tube, no antibiotic was applied, and ciprofloxacin served as the gold standard. MIC was defined as the minimum concentration necessary to inhibit bacterial growth (Shoaib et al., 2016) [19].

RESULTS AND DISCUSSION

PHYTOCHEMICAL SCREENING

Metabolite classes represented in *Artemisia santolinifolia* (Table 1) include terpenoids, tannins, phenolics, flavonoids, carbohydrates, and steroids, and they may be found in the plant's root, leaves, and stem. Proteins are absent from the root, saponins, leaves, and stem of *A. santolinifolia*, suggesting the herb may have medicinal use. Alkaloids, flavonoids, saponins, tannins, and steroids were identified as active chemical components responsible for antifungal action following a phytochemical analysis of the composition of the aqueous extracts. Flavonoids and saponin, two chemicals found in abundance in barley leaves, have potent antioxidant capabilities. As a co-factor in glucose metabolism and insulin level optimization, magnesium is also present. All synthetically generated medicinal plants contain the antibacterial flavonoids since they all have a carbonyl group. Today's phenolic test confirms that Artemisia species contain phenolic compounds. Tannins isolated from medicinal plants with antifungal and antibacterial action are likely to have other therapeutic applications as well. The soapy qualities of the unusual class of glycosides known as saponins have led to their widespread acceptance as an effective antifungal medication [17-19].

Table 1. Phytochemical screening of Artemisia santolinifolia.

Phytochemicals	Leaves	Stem	Root
Terpenoids	++	++	++
Tannins	++	++	++
Phenolics	++	++	++
Saponins	++	++	++
Flavonoids	++	++	++
Proteins	--	--	--
Carbohydrates	++	++	++
Steroids	++	++	++

ANTIOXIDANT ACTIVITIES

The DPPH assay and the ABTS assay were used to evaluate the antioxidant activity of the whole plant, including the root, the leaves, and the stem. The use of ascorbic acid as a reference point. Table 2 displays the results of the antioxidant activity tests using DPPH and ABTS. The IC50 table shows that the highest reaction to AsL is 51.05%.

Results from tests on the antioxidant DPPH activity at varying concentrations show that AsS has the highest DPPH% inhibition (80.451.84) at 1000 g/ml, while both AsL (77.111.14) and AsR (77.231.66) also exhibit high DPPH activity at this concentration. The DPPH activity

of AsS at 62.5 g/ml is lowest (62.291.11), while that of AsR at the same concentration is highest (49.191.32). Percent inhibition by ABTS was also measured across concentrations, with the greatest values found in AsS (79.341.19) and AsL (78.231.09) and the lowest value found in AsR (45.691.11). The highest mean value for ABTS was found to be (89.16 g/ml, 70.56 g/ml), and the lowest ABTS activity measured was (68.14 g/ml) (Figure 1), while the highest activity for DPPH was found to be (61.31 g/ml), and the lowest noted value was (51.05 g/ml).

Table 2. Antioxidant Assay of *Artemisia santolinifolia* with IC_{50} value.

Name	Code	Conc (µg/mL)	DPPH Percent inhibition	IC_{50} (µg/mL)	ABTS Percent inhibition	IC_{50} (µg/mL)
Artemisia santolinifolia	AsL	1000	77.11±1.14	51.05	78.23±1.09	68.14
		500	72.19±0.84		74.83±1.10	
		250	67.65±0.46		68.51±1.14	
		125	64.53±1.34		62.61±1.74	
		62.5	57.78±0.45		56.21±1.34	
Artemisia santolinifolia	AsR	1000	77.23±1.66	61.31	77.12±1.05	89.16
		500	70.39±1.01		71.34±1.34	
		250	66.52±1.41		63.45±1.87	
		125	59.21±1.57		54.78±1.21	
		62.5	49.19±1.35		45.69±1.11	
Artemisia santolinifolia	AsS	1000	80.45±1.84	53.61	79.34±1.19	70.56
		500	78.17±1.04		70.24±1.86	
		250	72.31±0.66		68.29±1.37	
		125	68.25±0.57		61.19±1.39	
		62.5	62.29±1.11		59.24±1.37	
Ascorbic acid	AsA	1000	86.50±0.00	<1	85.37±0.87	<1
		500	86.33±0.16		84.52±0.22	
		250	86.23±0.14		83.67±1.39	
		125	85.00±0.28		82.09±1.31	

		62.5	84.00±0.28		80.11±1.01	

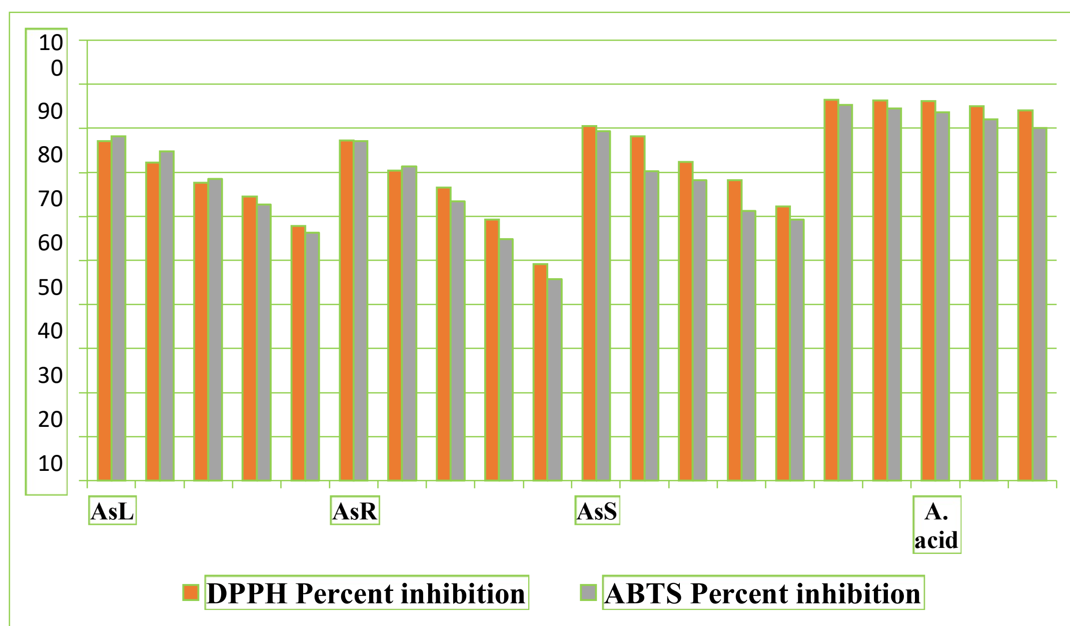

Figure 1. Antioxidant activities of *Artemisia santolinifolia.*

ANTIBACTERIAL ACTIVITIES

Agar well diffusion testing was used to see how well crude extract from different regions of chosen species fared against both gram-negative and gram-positive bacteria. Plates were made with gram-negative and gram-positive bacteria at three different concentrations (25 ml, 50 ml, and 100 ml) and the average zone of inhibition outside of the wells was recorded for each treatment. The MIC values for AsL against gram-positive bacteria, B. subtalis and S. aureus, at 50 g/ml are 23.21 1.14 and 29.13 1.18, respectively; against gram-negative bacteria, P. aeruginosa, they are 25.35 1.33 and 29.18 1.72, respectively. Inhibition zone means (n=3) for standard Ampicillin and Ciprofloxacin were 29.091.78 (gram-positive), 38.151.51 (gram-negative), 33.120.96 (gram-positive), 31.352.01 (gram-negative), 35.121.39 (gram-negative), and 32.011.05 (gram-positive).

L. elasticus extracts demonstrate that all organisms are inactive in organic solvents up to 200 g/ml. Both gram-negative and gram-positive organisms exhibit activity at a concentration of 25 g/ml in extracts of ethanol and methanol. When compared to other solvents, ethanol and methanol seem to be the most effective for all organisms examined (Krishnaveni et al., 2016). For maximum zone of inhibition against all bacteria, a greater concentration of essential oil (80 L/well) is required during anti-microbial activity. Essential oil of *A. maritima Linn* has inhibitory capacity to growth of both gram-negative and gram-positive bacterial strains, as evidenced by its maximum and minimum zone of inhibition concentration against

Pseudomonas fluorescence (MTCC-664) and Bacillus subtilis (MTCC-2451). Because of Artemisia genus previous studies and present results, it can be inferred that *A. maritima Linn* is an aromatic and higher altitude medicinal plants, numerous grams positive and gram-negative bacterial strains work as a substantial anti-microbial agent. Artemisia. dracunculus Essential Oil had an antibacterial MIC of 6.25 mg/mL against Staphylococcus aureus and Bacillus subtilis. While E. coli VKPM-M17 had a MIC of 50 mg/mL when exposed to EO components, P. aeruginosa's MIC was 150 mg/mL. The dhp-pUCl8 strain of antibiotic-resistant E. coli showed a low MIC value of 6.25 mg/mL against the O (Table 3).

Bacteria were killed by the action of. The MIC for the oil component was found to be 1.56 milligrams per milliliter for the yeasts tested. Conclusions drawn from this study indicate that A. dracunculus EO has potential as an antibacterial natural agent in the cosmetic, medical, and nutritional industries. Recent research has shown that gram-positive bacteria are especially vulnerable to this compound. To kill S. aureus and B. subtilis, 6.25 mg/mL of A. dracunculus EO was required. When tested against E. coli, the MIC value of the oil was 50 mg/mL, but when tested against P. aeruginosa, the MIC value was 150 mg/mL, indicating that P. aeruginosa was resistant to the EO components. The gram-negative microbe most susceptible to the examined oil was antibiotic-resistant E. coli, with a MIC value of 6.25 mg/mL. Both yeasts examined had a minimal inhibitory concentration (MIC) of 1.56 mg/mL when exposed to oil components. Essential oils were found to have a bactericidal effect in this investigation, and the MIC values found were found to be acceptable and effective.

Against Gram-negative and Gram-positive bacteria, the table 4 shows the minimum inhibitory concentration (g/ml) of crude extract from different sections of selected species. From personal experience, I can say that AsL has low-concentration inhibitory potentials against all studied bacteria. The extract AsL showed MIC of 12.5µg/ml for B. subtilis which is gram positive bacterium, 50µg/ml for gram positive bacteria S. aureus and 37.5 µg/ml for gram negative bacteria *P. aeruginosa* [20-26].

Table 3. Antibacterial activities of *Artemisia santolinifolia.*

		Zone of inhibition (mm)		
Crude samples	Concentration (µg/ml)	Gram positive Bacteria		Gram-negative Bacteria
		B. subtilis	*S. aureus*	*P. aeruginosa*
AsL	25	19.32±2.61	25.11±2.61	23.09±0.91
	50	23.21±1.14	29.13±1.18	25.35±1.33
	100	21.09±1.82	28.22±1.71	29.18±1.72
	25	13.15±1.71	16.19±1.48	15.28±1.41

AsR	50	15.13±2.11	16.71±1.72	16.09±2.08
	100	19.11±1.51	18.19±1.51	21.38±1.14
	25	10.81±1.71	6.18±1.84	7.09±2.13
AsS	50	13.39±2.21	8.90±0.58	8.27±1.04
	100	14.65±1.91	8.27±1.41	8.77±1.26
Ampicillin	10	29.09±1.78	38.15±1.51	33.12±0.96
Ciprofloxacin	10	31.35±2.01	35.12±1.39	32.01±1.05

All values are taken as mean ±SEM (n=3)

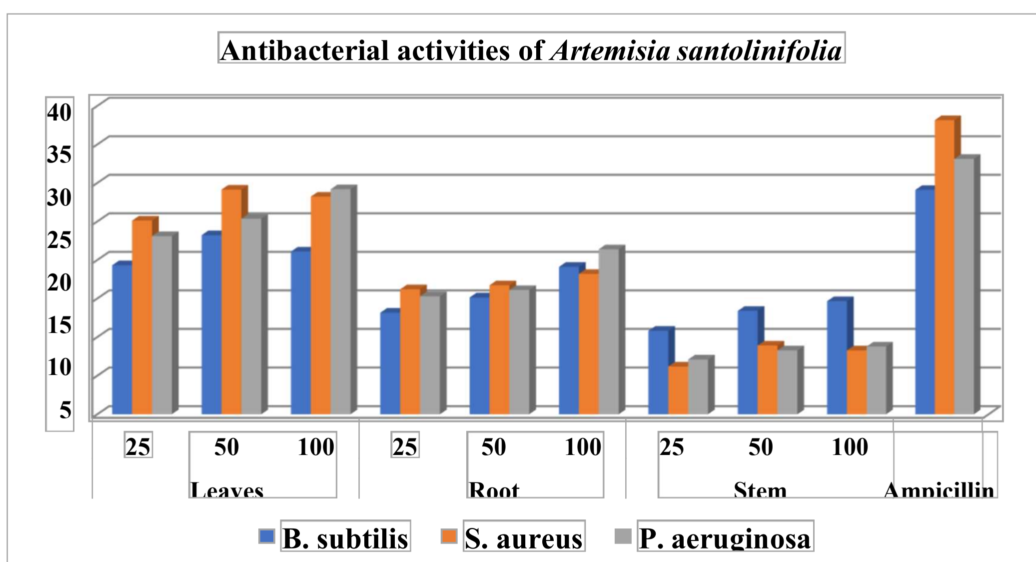

Figure 2. Antibacterial activities of *Artemisia santolinifolia.*

Table 4. The antibacterial activity MIC of selected Artemisia species crude extract.

Crude extract samples from various parts of selected species	MIC (µg/ml)		
	Gram-positive Bacteria		Gram-negative bacteria
	B.subtilis	*S.aureus*	*P. aeruginosa*
AsL	12.5	50	37.5

AsR	62.5	50	25
AsS	100	100	>125
Ciprofloxacin	6.25	6.25	6.25

CONCLUSIONS

It was determined through this research that both simple and complex disorders can be treated with Western medicine. For the treatment of common ailments such a cough, cold, fever, bites, headache, skin illness, or tooth infection, many residents of the study region continue to rely on medicinal plants. The quality of therapy is enhanced by the positive interactions between patients and the experts of medicinal plants (Hakims). Conventional medical practice was antiquated at best nowadays. The next generation may soon lose this treasure of knowledge due to a lack of interest and a trend toward migration to urban centers in search of better employment opportunities. The conventional medical system must be captured and preserved through careful recordkeeping. The findings of this investigation provide empirical support for herbal medicine's time-honored history of use. However, further research is needed to identify the active ingredients in therapeutic plants. If the active components of Artemisia species can be isolated, the plant's antibacterial properties may be improved. The identification of various useful compounds following phytochemical study further demonstrated the significance of medicinal plants.

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