Section A-Research paper
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Standardization and potential antimicrobial activity of *Gorakhmundi Sphaeranthus indicus* Linn

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Abstract

Background: *Sphaeranthus indicus Linn*, in the Ayurvedic medical system, is used in different dosages and preparations to treat a variety of ailments, including tuberculosis, lung conditions like bronchitis and asthma, indigestion, spleen disorders, elephantiasis, leukoderma, vomiting, urinary discharge, hemicrania, epileptic convulsions, blood purifier, as a poultice for rheumatic pain, aphrodisi. **Objective:** To study and standardization and anti-microbial activity of Gorukmandi. **Methods:** After collecting herbs, the extract of Gorukmandi was prepared hydroalcoholic procedure. Then organoleptic, Physico-chemical analyses were done, along with the Standardization method – a chemical with HPTLC method, Heavy Metal Analysis, Anti-Microbial Activity, Total antioxidant by DPPH method, Total phenolic, Total Flavonoid, were performed. **Results:** The HPTLC studies have shown that it is more versatile than ordinary TLC methods, Heavy Metal analysis showed a minimum presence of metals, antioxidants, polyphenols, and flavonoids. The anti-microbial activity shows that the hydroalcoholic extracts inhibited good antimicrobial activity against all tested fungi and bacteria **Conclusion:** It is a conclusion that the standardization and potential antimicrobial activity of the extracts from Gorukmundi against tested bacteria and fungi. Further investigation work is to characterize the bioactive compound and other values for fixing standards for this plant.

Keywords: Anti-microbial, HPTLC analysis, Hydrohalic, Phytochemical.

Introduction:

Herbal medicines have been developed and are in use for decades. The Ayurveda, an oldest Indian traditional system depicts the prehistoric Indian therapeutically benefits of several herbs. The geographical and climatic varication enables India to be a nation of diverse and abundant flora. This Ayurveda and scientific research have developed and enabled the reconstruction and advancement of traditional medicines. As many plant extracts have been developed and researched for various treatments so far. *Sphaeranthus indicus* Linn. is one of the common most Indian traditional medicinal plants used for a variety of illnesses. It is mostly grown in rice fields, arid waste areas, and cultivated plains. It is native to India, Sri Lanka,

Africa, and Australia. It is also known as *Sphaeranthus hirtus* Willd. and *Sphaeranthus mollis* Roxb.

In the Ayurvedic medical system, it is used in different dosages and preparations to treat a variety of ailments, including tuberculosis, lung conditions like bronchitis and asthma, indigestion, spleen disorders, elephantiasis, leukoderma, vomiting, urinary discharge, hemicrania, epileptic convulsions, blood purifier, as a poultice for rheumatic pain, aphrodisia [1]. The plant's seeds and roots have demonstrated anthelmintic action [2]. It also has been pharmacologically or traditionally found to be an effective drug for the treatment of various disorders, as a hepatoprotective agent [3], antihelmintic agent [4], antigout activity [4], antitussive agent [5], antioxidant agent [6] [7], as a renal protective agent [8], as immunostimulant agent [9], immunomodulatory [10], antidiabetic agent [11] anxiolytic [12], macrofilaricidal [13], antimicrobial [14], insecticidal [15], antifungal [16], and hypoglycaemic [17] activities. Very recently new eudesmanolide glycoside has been reported from S. indicus [18].

This plant was chosen for further study by the authors due to its diverse spectrum of biological activity. According to the medical knowledge gathered from the tribal healers, the herb is traditionally used to cure fever, cough, and skin conditions [2]. When combined with whey, the bark has been proven to be effective at treating piles [19]. It has been discovered that the Flowers have tonic effects. To treat a cough, milk, and leaf juice are cooked together. Sugar candy is also recommended. The entire plant's aqueous extract was discovered to be poisonous to American cockroaches [20].

Materials and method:

Collection of Plant: Gorakhmundi (*Sphaeranthus indicus Linn*) plant collection from Sikkim India for research study and Extract as Figure:01 and Figure:02.

Extraction – a hydroalcoholic procedure

In this study, the hydroalcoholic protocol is used for the test solution extraction, where 2.5g of dry Gorkhmundi sample is added with 50mL of methanol in a beaker, and refluxed for 1 hour. The solution is then cooled and filtered using a 0.45-micron membrane filter.

Organoleptic study:

Organoleptic refers to something that can be sensed through the senses of smell, appearance, taste, touch, odor, etc. [22]. The organoleptic characteristics of dried materials can be evaluated in many methods, including chemical or microscopic analysis and direct sensory perception. Organs of sense can be used to perform the organoleptic assessment, identifying certain specific properties of the substance that can be seen as the first step towards establishing identification and degree of purity. [23]. The organoleptic investigations (condition, color, odor, taste, texture, and nature) were performed and tabulated in Table 1.

Physico-Chemical Analysis:

Loss on drying (LOD): In the dried glass stopper shallow weighing bottle, add 1.0g and noted the total weight of the glass stoppered bottle with a sample in it (A). The loaded bottle was placed in the drying

chamber (hot air oven) without the stopper, placing the stopper in the same chamber. The sample was dried at 105 + 2oC for about 1 hour. After one hour the lid was placed and the bottle was cooled and then weighed (B). The percentage loss of drying was calculated using the following formula

$$\% \text{ loss of drying} = \frac{(B - A)}{\text{Wt of Taken}} x100$$

pH Value: 1.0 g of sample was transferred into 100mL beaker and 100 water is added and mixed well for about 1.0 minutes. The pH meter electrode was placed into the beaker and 3 readings were taken, the average of which was noted as the pH value of the sample.

Water soluble extractive for Extract and powder: In a 250mL conical flask 5.0 g of sample was transferred and 100 mL water and was placed on a shaker for 6 hours and then allowed to stand for 18 hours, and then filtered and evaporated 25mL of the filtrate to dryness in a weighed evaporating dish. Further dried at 105oC to a constant weight and weigh. Then the percentage of water-soluble extractives was calculated using the following formula.

Wt. taken (A): a g in 100 mL, 25 mL, taken

i.e., $\frac{25xa}{100}$

$$\% \text{ Of water-soluble extractive} = \frac{\text{Wt.of residue}}{Wt.of\ sample\ (A)} x100$$

Alcohol soluble extractive: In a 250mL conical flask 5.0 g of sample was transferred and 100 mL alcohol of specified strength was placed on a shaker for 6 hours and then allowed to stand for 18 hours, and then filtered and evaporated 25mL of the filtrate to dryness in a weighed evaporating dish. Further dried at 105oC to a constant weight and weigh. Then the percentage of alcohol-soluble extractives was calculated using the following formula.

Wt. taken (A): a g in 100 mL, 25 mL, taken i.e., $\frac{25xa}{100}$

$$\% \text{ Of alcohol-soluble extractive} = \frac{\text{Wt.of residue}}{Wt.of\ sample\ (A)} x100$$

Total ash: To a pre-weighted crucible 1.0g of air-dried sample was added and then again weighed. The sample was then incinerated gently at 450 ^{o}C, until free from carbon. And then weighed. Then the percentage of total ash was calculated using the following formula.

$$\% \text{ Of Total Ash} = \frac{\text{Wt.of Ash}}{Wt.of\ sample} x100$$

Acidic Insoluble Ash: The Ash obtained from the total ash was taken to determine the acid-insoluble ash content. 10% of diluted HCl was added and boiled in a water bath for 10 min, and filtered through the ashless filter paper. The soluble matter was washed with hot water. The insoluble matter of ash was poured into the crucible (one used for total ash) and dried on the hot plate. And then in France, it was ignited for an hour at 800^{o}C. The crucible is then cooled in a desiccator and weighed. Then the percentage

of Acidic Insoluble Ash was calculated using the following formula.

$$\% \text{ Of Acidic Insoluble Ash} = \frac{\text{Wt.of acidic insoluble ash}}{Wt.of\ sample} x100$$

Standardization method – a chemical with HPTLC method:

The extract was filtered using Whatman filter paper No.1. Extract residue was re-dissolved in 1ml of chromatographic grade alcohol, which was used for sample application on pre-coated MERCK-TLC/ HPTLC silica gel $60F_{254}$ aluminum sheets. Several solvent systems were obtained in the solvent of Chloroform: Methanol: Glacial acetic acid (9:1:0.2 v/v). Application of bands of 10ul extract was carried out using the spray technique by using Anisaldehyde-sulphuric acid reagent, which was prepared by mixing 0.5mL Anisaldehyde, 10 mL Glacial acetic acid, 85 mL Methanol, and 5 mL Sulphuric acid (98%). The sample was applied in duplicate on pre-coated silica gel $60F_{254}$ aluminum sheets, considering the start point 10 mm on the Y axis and 80 mm as the end point from the plate, with the help of CAMAG Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed by WIN CATS software. The chromatogram was generated in CAMAG TLC twin traversing the chamber for 30 minutes following the application of spots. The drying was done through TLC Plate Heater preheated at 100+ 5^oC for 3 minutes. The air-dried plates were viewed in ultraviolet radiation to mid-day light. A densitometer was used to scan the chromatograms, at 254nm, 366nm, and 540 nm after spraying with anisaldehyde sulphuric acid The Rf values and fingerprint data were recorded by WIN CATS software.

Heavy Metal Analysis:

The 10-ppm working Standard solution was prepared, by adding 1mL of standard stock solution (1000 ppm) in a 100mL volumetric flask and made up to the volume with Type-1 water. From this working standard solution 2ppb, 5 ppb, 10ppb standard solutions were made by adding 100 µL, 500 µL, and 1 mL of10 ppm working standard solution in respective 100 mL volumetric flask and made up to the volume with Type-1 water, respectively. The sample solution was prepared by adding 0.4g sample in PFA(Perfluoroalkoxy) Teflon vessels and adding 5mL trace metal grade Nitric acid (HNO_3). The calibration curve was plotted on ICP-MS, and the metals were detected in samples using the calibration curve.

Antimicrobial Activity

Bacterial culture: The freshly prepared slant of *S. aureus, P. aeruginosa, E. coli, B. subtilis, C. albicans & A. brasiliensis* was used, and washed the slant by using 10 mL of sterile Normal saline solution.

Media Preparation-Muller Hinton Agar (MHA) was used for determining the activity of *S. aureus* (ATCC6538), *P. aeruginosa* (ATCC 9027), *E. coli* (ATCC8739) & *B. subtilis* (ATCC 6633). Sabroud Dextrose Agar (SDA) was used for determining the activity of *C. albicans* (ATCC 10231). Potato Dextrose Agar (PDA) was used for determining the activity of *A. brasiliensis (ATCC 16404)*. Media was prepared as per the Manufacturer's Instructions. The media was then autoclaved

at 121˚C temp. & 15 lbs pressure for 20 minutes.

Sample Preparation: Test samples were weighed 5 gm and transferred into different 150 ml labeled flasks. 2 ml of Dimethyl sulfoxide, 9 ml of methanol & 9 ml of water were added in it & sonicated for 10 mins. After sonication, samples were refluxed in the water bath at 90˚C for 1 hr. Samples were filtered with Whatman filter paper & concentrated up to 5 ml. These samples were used for anti-microbial activity.

Testing Procedure: For antimicrobial activity 10µl of bacterial culture was pipetted into MHA flasks, and 10µL of yeast culture was pipetted into the SDA flask. 10µL of fungal culture was pipetted into the PDA flask. Mixed it slowly, labeled the plates & then poured 25 ml of media into a sterile measuring cylinder. After solidifying, wells were drilled into agar plates containing inoculums using a sterile borer. 100 l of each sample, along with a blank, was then put into the appropriate wells. Upon the diffusion of samples, the MHA plates were incubated in a Bacteriological incubator at 35°C for 24 hours & the SDA & PDA plates were incubated in a Biological Oxygen Demand incubator at 25°C for 48 hours. The extract was tested against two Gram-positive and two Gram-negative bacteria, as well as two fungi. By measuring the zone of inhibition, which included the well's diameter, after the incubation time, antimicrobial activity was discovered.

Microbial Limit Test:

Sample preparation of 1 gm/mL or 10 gm/mL samples were taken in 10 mL/100 mL sterile Soyabean casein digest broth. Samples were mixed using a cyclomixture.

For microbial limit, the test Pour –plate method was used

Total microbial plate count: From the prepared sample, 1 mL of the sample was pipetted into two different sterile Petri plates and labelled properly. Autoclaved Soyabean casein digest agar medium was cooled for about 40°C and approximately 25 mL of media was poured into each label plate and rotated slowly for proper mixing. And allowed the plates to solidify then incubated at 30° to 35°C for 3-5 days. The colonies from two plates were counted which have not more than 250 colonies and the mean was taken. The colony-forming unit was counted by the following formula.

Colony forming unit (cfu):

$$\frac{\text{No. of colonies x dilution}}{\text{Weight of sample}}$$

Total yeast & mold count: From the prepared sample, 1 mL of the sample was pipetted into two different sterile Petri plates. And labeled. Autoclaved medium of Sabouraud dextrose agar was cooled for about 40°C and approximately 25 mL of media was poured into each label plate and rotated the plates slowly for proper mixing. And allowed the plates to solidify and then incubate at 20° to 25°C for 5 days. The colonies from two plates were counted which have not more than 20 to 25 colonies and the mean was taken. The colony-forming unit was counted by the above-mentioned formula.

Test for Specified Organisms:

Escherichia coli: 0.1 mL sample was pipetted and transferred the same to 10mL

of MacConkey Broth and incubated at 42° to 44°C for 24 to 48h. The Subculture on a plate of MacConkey Agar was made and incubated at 30° to 35°C for 18 to 72h. The growth of pink, non-mucoidal colonies indicated the possible presence of *E.coli* which was confirmed by identification tests.

Salmonella: 0.1 mL sample was pipetted and transferred the same to 10 mL of *Rappaport Vassiliadis Salmonella* enrichment Broth and incubated at 30° to 35°C for 24 to 48h. The subculture on a plate of Wilson and Blair's BBS agar was made and incubated at 30° to 35°C for 24 to 48h. Green colonies with black centers developed and in 48h the colonies become uniformly black. Salmonella might be present in colonies that are encircled by a black zone and that are metallic in appearance. Additionally, formed red colonies with or without black cores suggest the potential presence of Salmonella when subcultured on XLDA plates and incubated at 30 to 35°C for 24 to 48 hours. This was validated by identification tests.

Pseudomonas aeruginosa: A loopful sample was obtained from the prepared sample, streaked on a plate of Cetrimide agar, and incubated for 18 to 72 hours at 30 to 35 degrees Celsius. Pseudomonas aeruginosa may have been present in the colony because of its greenish hue, which was verified by identification tests.

Staphylococcus aureus: From the prepared sample, a loopful sample was taken and streaked on a plate of Mannitol salt agar and incubated at 30 to 35°C for 18 to 72h. Yellow or white colonies with yellow zones indicated the possibility of *S.aureus*., which was confirmed by identification tests.

Negative Control: To test the sterility of the medium and the diluent, (without organisms) is known as the negative control. There should not be any growth of microorganisms in this control.

Total antioxidant by DPPH method:

1 g of sample was taken and extracted in 20 mL of 50 % methanol in a conical flask. Next day filtered with Whatman filter paper No. 1 and volume made up to 50 mL with 50 % methanol. This is a Stock solution. From this stock solution, 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL, and 1.2 mL was taken in different volumetric flask and volume made up to 100 mL with 50 % methanol to get working solutions with a concentration of 80 µg, 120 µg, 160 µg, 200 µg, 240 µg respectively. The working solutions thus obtained were used for the Antioxidant study.

Total phenolic:

The standard solution was prepared by adding 100mg standard Gallic acid and 75 mL of 50& methanol solution and sonicated for 15 min (until the standard is completely dissolved). The solution volume was made up to 100 mL by adding 50% methanol solution. From which 5mL solution was transferred and made up to 50 mL by adding DM water.

The sample was prepared using 500mg of extract/ powder in an iodine flask to which 75 mL 50% methanol solution was added and reflexed in a water bath for30min, cooled, and filtered with Whatman filter paper in a volumetric flask. The solution is made up to the volume of 100 mL with 50%

methanol solution, from which 5mL of the solution was taken and made up to 50mL using DM water.

The Assay was performed by pipetting 2 mL of standard and sample solution in a 2separate volumetric flask (50 mL). 20 mL of water, 2.5 mL of Folin-Ciocalteu reagent, and 5 mL of 30% Na_2CO_3 were added and allowed to stand for 30mins. The volume of the solutions was made up to 50mL using DM water, Shaked well, and allowed to stand for 20min. The absorbance of the standard and sample was taken using a UV spectrophotometer at 750nm using DM water as blank. Then the percentage of Total Polyphenols was calculated using the following formula.

$$\%\ of\ Total\ Polyphenol = \frac{T}{S} x \frac{Cs}{Ct} x \frac{P}{(100 - LOD)} x100$$

Where: T = absorbance of test solution; S = absorbance of standard solution; Cs = concentration of standard solution; Ct = concentration of test solution; P = Purity of standard; LOD = loss on drying of extract.

Total Flavonoid:

1g of weighed sample was taken and 60mL of methanol was added, shaken for 1 hour on a shaker, and allowed to stand for 18 hours. The next day it is then filtered and the residue is used and given for washing with 50mL methanol and repeated. All the methanolic filtrate was combined and concentrated to 10 mL. then this 10 mL solution was added dropwise with continuous string to the beaker containing 100mL diethyl ether. And kept stand for 10 min. and filtered with Whatman filter paper and collected the filtrate was in a pre-weighed porcelain dish and the solution was evaporated in a water bath to dryness and further dry at 100°C in an oven to a constant weight. Then the percentage of Total Flavonoid was calculated using the following formula.

$$\%\ \text{Of Total Flavanoids} = \frac{\text{Weight of residue}}{Wt.of\ sample\ taken} x100$$

Results and Discussion

Organoleptic Analysis:

The organoleptic analysis reported Light Brown colored powder with characteristic odor and taste.

Parameters	Result
Description	Light Brown colored powder
Odor	Characteristic
Taste	Characteristic

Table 1: Results of Organoleptic Analysis

Physico-Chemical Analysis:

The Physico-chemical Analysis is tabulated in TableNo.2 below:

Parameters	Result
Loss on Drying	1.89%

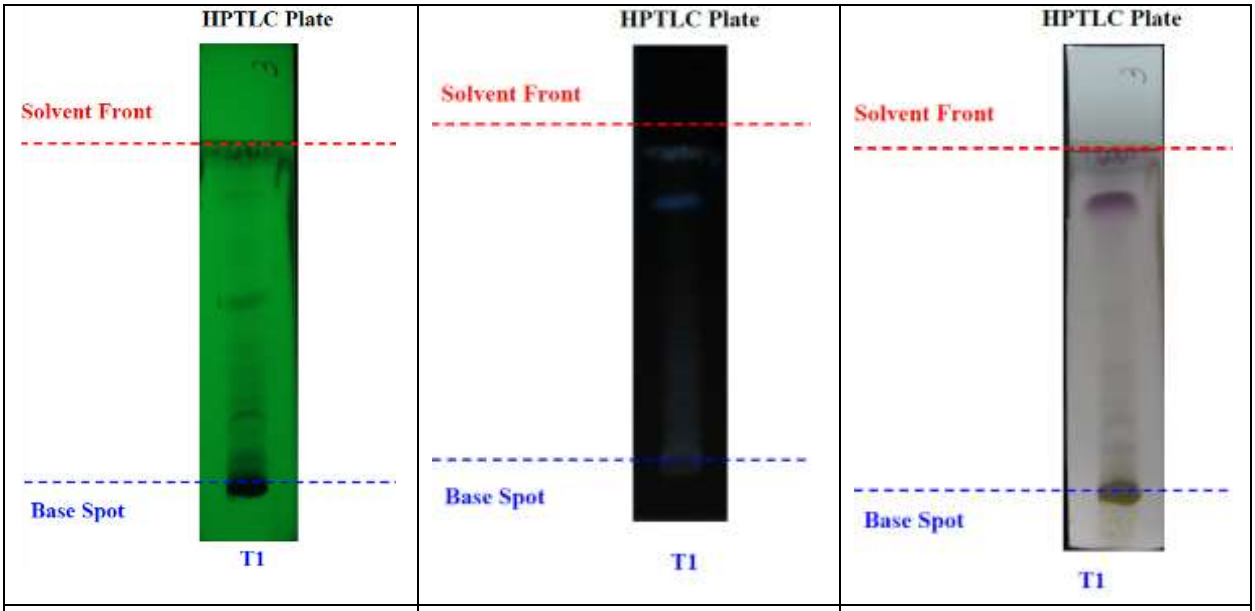
pH (1%)	4.67
Water Soluble Extractive	92.45%
Alcohol Soluble Extractive	82.67%
Total Ash	6.29%
Acid Insoluble Ash	1.47%

Table 2: Results of Physico-Chemical Analysis

HPTLC results:

In this study, the HPTLC fingerprinting of the alcoholic extract revealed thirteen spots for HPTLC Chromatogram at 254 nm at the following Rf values 0.18, 0.23, 0.26, 0.31, 0.33, 0.35, 0.41, 0.55, 0.61, 0.67, 0.71, 0.85, 0.90. HPTLC Chromatogram at 366nm revealed twelve spots at the following Rf values 0.11, 0.15, 0.18, 0.26, 0.31, 0.33, 0.35, 0.41, 0.50, 0.55, 0.61, 0.85. And at 540 nm revealed seven spots at the following Rf values 0.15, 0.23, 0.26, 0.33, 0.35, 0.55, 0.85, and the purity of the sample was confirmed by comparing the absorption spectra at the start, middle, and end positions of the band. An essential tool for determining the quality of botanical materials is HPTLC. It makes it possible to analyze many different substances quickly and affordably. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots were well resolved. Though further work to characterize the other chemical constituents and quantitative estimation with marker compounds is also necessary these data can also be considered along with the other values for fixing standards to this plant.

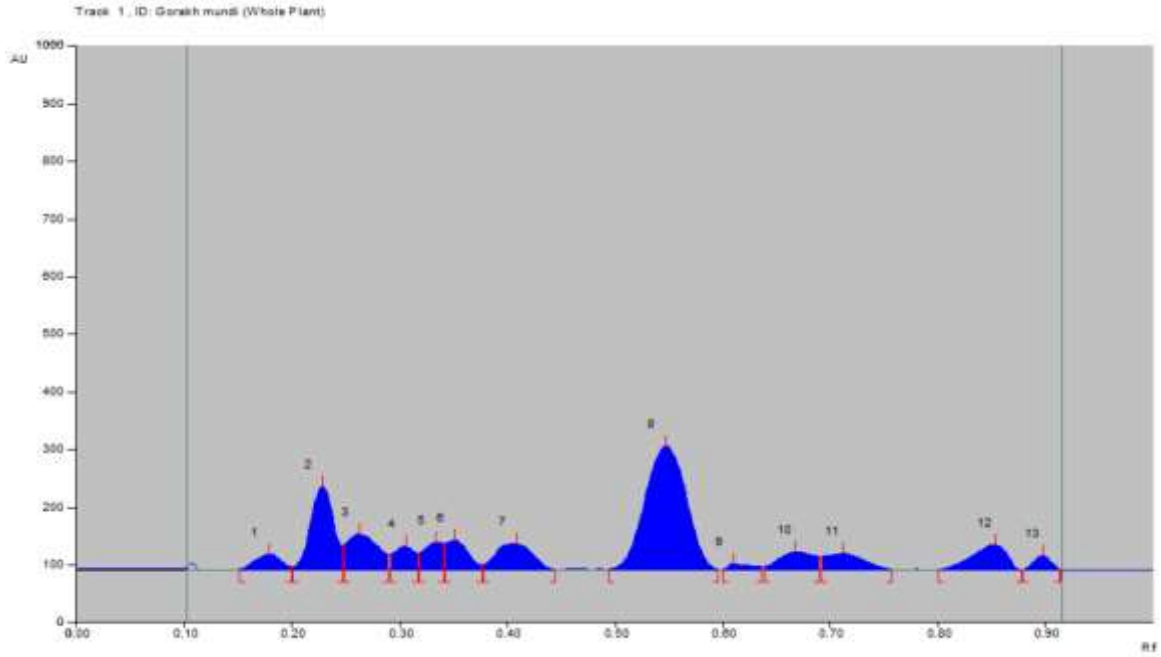
The corresponding HPTLC chromatograms are presented in Fig. 1 and 2, for the respective densitometer



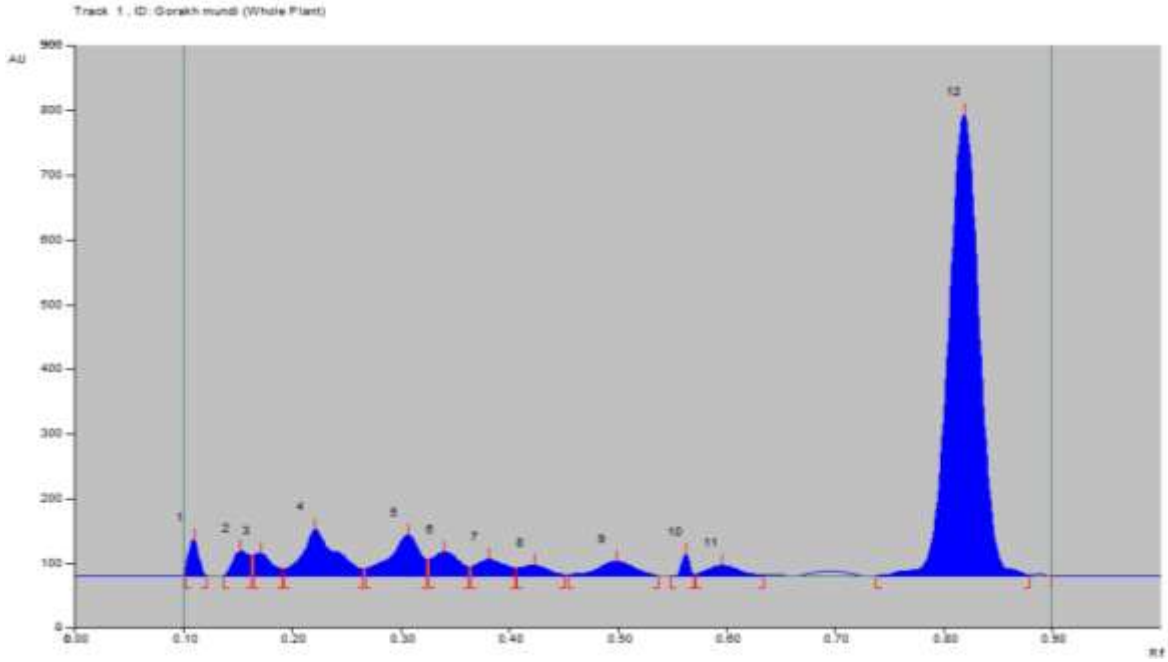
@ 254	@366	@540

Figure 3: HPTLC chromatogram of the sample at 254nm, 366nm, and 540nm in alcoholic

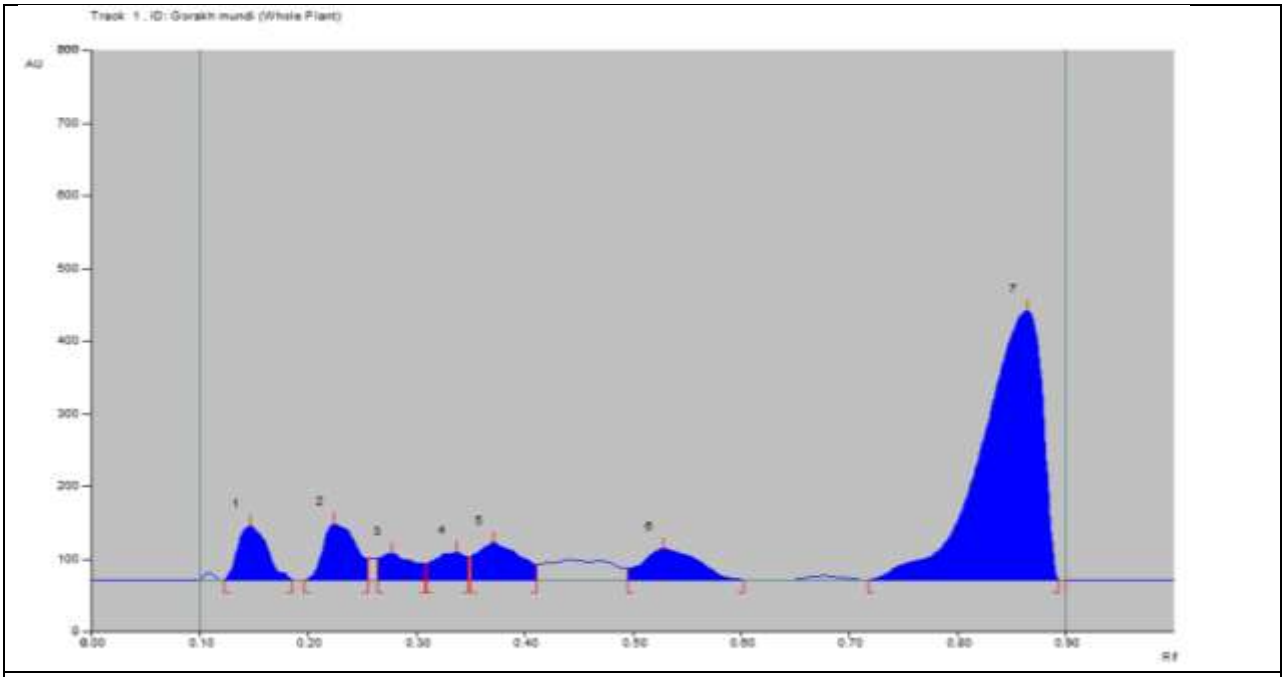
extract (anisaldehyde sulphuric acid sprayed data).



@254nm



@366 nm



@540

Figure 3: HPTLC chromatogram of alcoholic extract at 254nm, 366nm, and 540nm showing different peaks (bands) of phytoconstituents.

Heavy Metal Analysis:

The Heavy Metal Analysis is tabulated in TableNo.3 below:

Parameters	Result	Limit as per API
Lead	0.008 ppm	NMT 10 ppm
Cadmium	0.001 ppm	NMT 0.3 ppm
Mercury	0.002 ppm	NMT 1 ppm
Arsenic	0.005 ppm	NMT 3 ppm

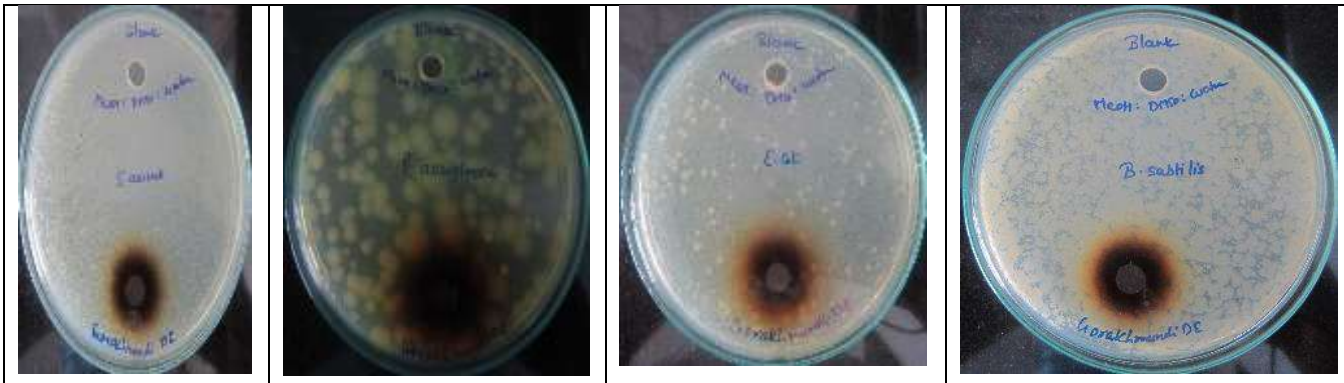
Table 3: Results of Heavy Metal Analysis

Anti-microbial activity:

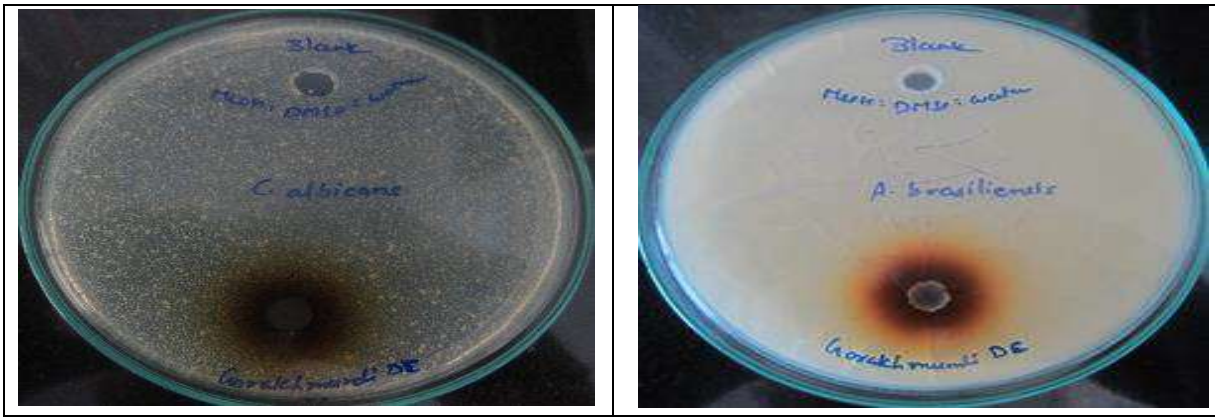
The extracts from A. officinalis were tested against two Gram-positive and two Gram-negative bacteria, as well as two fungi. The results, presented in Table 4, show that the methanolic extracts exhibited good biological activity against all tested fungi and bacteria. The most sensitive microorganism against extracts was *Staphylococcus aureus* with inhibition zones of 17mm. Other microorganisms such as *Pseudomonas aeruginosa* with inhibition zones for 12mm, *Escherichia coli* 10mm, *Bacillus subtilis* 11mm, *Candida albicans* 10mm, *Aspergillus brasiliensis* 10mm, respectively were found to be moderately sensitive to the extracts with

inhibition zones. It is conceivable that the antimicrobial property of the hexane extracts from A. officinalis might be ascribed to its high content of fatty acid compounds.

Name of Sample	Zone of Inhibition (mm)					
	S. aureus	***P. aeruginosa***	***E. coli***	***B. subtilis***	***C. albicans***	***A. brasiliensis***
Blank	NZI	NZI	NZI	NZI	NZI	NZI
Gorukmandi DE	17 mm	12 mm	10 mm	11 mm	10 mm	10 mm
Table 4: Anti-microbial & anti-fungal activity of Gorukmandi DE extraction						



S. aureus	***P. aeruginosa***	***E. coli***	***B. subtilis***
Figure 4: Anti-bacterial activity of Gorukmandi DE			
NZI: No Zone of Inhibition			



C. albicans	***A. brasiliensis***
Figure 4: Anti-fungal activity of Gorukmandi DE	

Microbial Limit test:

The extracts from A. officinalis were tested for microbial activity against four microbes through the plate-pour method. The results, presented in Table 5, showed the total microbial count of 74 cfu/g and the absence of yeast and mold count and tested microbes namely *Staphylococcus aureus, Salmonella sp., Pseudomonas aeruginosa, Escherichia coli.*

Sr No.	Parameters	Results	Limits as per API
1	Total Microbial Plate Count	<10 cfu/g	10^5 cfu/g
2	Total Yeast & Mould Count	<10cfu/g	10^3 cfu/g
3	*Staphylococcus aureus*	Absent	Absent/g
4	*Salmonella sp.*	Absent	Absent/g
5	*Pseudomonas aeruginosa*	Absent	Absent/g
6	*Escherichia coli*	Absent	Absent/g

Table 5: Microbial analysis of Gorukmandi DE

DPPH scavenging effect (%):

The DPPH showed the following results at different concentrations tabulated in Table 6. Of which the graph was plotted (figure5).

Concentration (µg)	DPPH Scavenging effect (%)
120	30.64
180	43.24
240	58.01
300	68.01

Table 6: Results of DPPH Scavenging effect at different concentrations

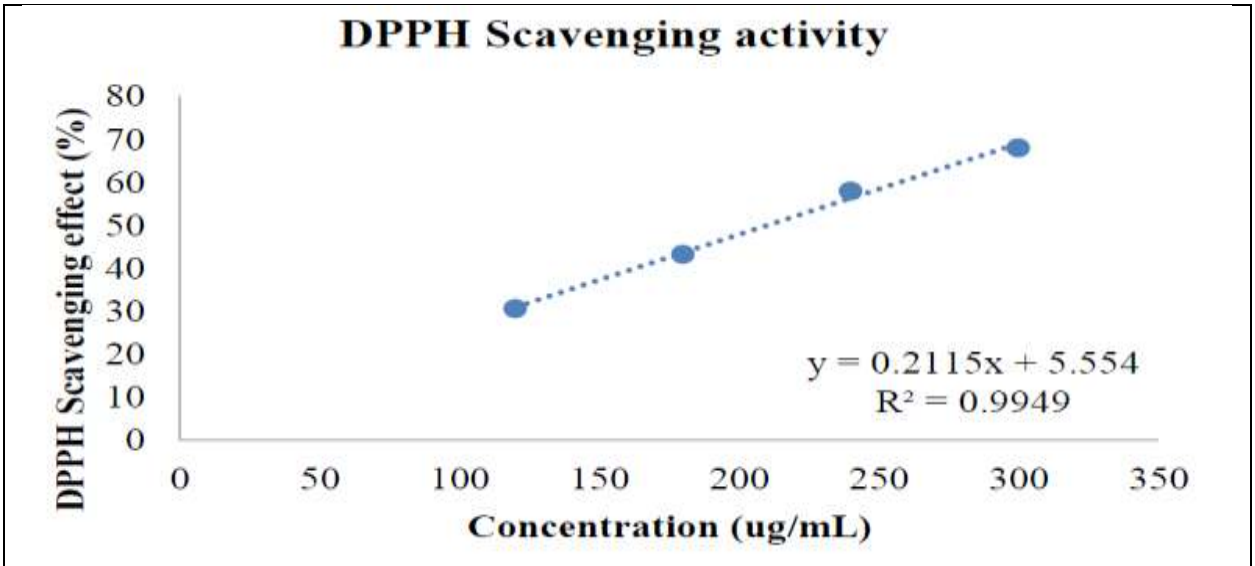


Figure 5: DPPH Scavenging Graphs

Total phytochemicals

The quantification of total phenols and flavonoids was carried out using a UV spectrophotometer. Phenols are considered to have strong antioxidant properties and possess anticancer, antimicrobial, and antiallergic properties. Flavonoids are considered the most common group of polyphenolic compounds in the human diet and are found in plants. The quantitative analysis of these compounds is very important to check the quality of the drug. For about 2.06% of polyphenols and 15.7% of flavonoids are observed in the extract.

The hydrophilic extracts' organoleptic analysis revealed a pale brown color. In another study when different plant sections were studied separately, it was found that flower extracts (benzene, chloroform, ethyl acetate, and acetone) were light brown to brown in color, while hexane extracts were pale yellow, and extracts from aerial portions were dark green to dark brown [2].

The health of individuals and communities depends heavily on phytomedicine, which is one of the most significant branches of traditional medicine worldwide. It is crucial to investigate medicinal plants, which have a folkloric reputation, in more depth to encourage the right use of phytomedicine and establish their potential as sources for novel medications [24]. Standardization is a crucial analytical technique used nowadays to guarantee the efficacy of herbal medicines. The quality of herbal medications is assessed using a variety of physicochemical characteristics. Physical and chemical characteristics in the current investigation include loss of drying, and pH value and to assess the drug's purity, it was determined how much of the substance was soluble in water, alcohol, total ash, and acidic insoluble ash. The results are listed in Table 2.

Contrary to readings from prior studies on ethanol and methanolic extract, which were discovered to be 8% and 0.05%, respectively, the current study revealed the water-soluble and acid-insoluble to be 92.45% and 1.47%, respectively [25]. These extractive values are most helpful for identifying medicine that has been used up or that has been tampered with.

It is helpful to analyze physicochemical characteristics like moisture content and ash values because they reveal the physiological and non-physiological states of ash (Mulla and Paramjyothi 2010)., which may be used to assess the likelihood of microbial development and, finally, contaminants or impurities. The ash values were utilized to look for any foreign materials, such as sand and soil or salts that were water soluble and stuck to the medications' surface. The ash values of various medications usually range significantly from one another, although for the same drug, the difference often stays within certain bounds. The presence of silica and high acid insoluble ash in the acid insoluble ash indicates the contamination with earthy elements. Higher levels of moisture in the drug sample may lead to hydrolysis of the medicine's active components, which lowers the drug's quality

and effectiveness. Both the drug's ultimate dryness and the pace of moisture elimination are crucial.

The sample's phytochemical analysis showed that the phenolic and flavonoid contents were 2.06% and 15.7%, respectively, as opposed to 2.1% and 8%, in some other research [25]. The sample contained 3.1% of its total weight in tannins. It is noted that the sample has the highest concentration of flavonoids, which illustrates the nutritional value and socio-economic value of the fruit of S. indicus.

Flavonoids, which are found in plants, are the most prevalent class of polyphenolic chemicals in the human diet [26]. Acting as antioxidants are the phenolic chemicals. Additionally, these substances are said to have antimicrobial, anti-inflammatory, anti-allergic, and anti-cancer properties. Plants synthesize derivatives of phenolic compounds, the most common kinds of phytochemicals, using the pentose phosphate, shikimate, and phenylpropanoid pathways [27]. These substances are secondary metabolites that play a crucial function in development and reproduction and provide defense against dangerous diseases and predators. Therefore, to assess the quality of the medicine, it is important to quantitatively analyze these components.

A logical approach for stronger and more effective quality control of natural goods is HPTLC fingerprinting, and HPLTC is an essential analytical tool in the separation, detection, and quantification of numerous kinds of natural products [28]. The Camag-HPTLC machine (Switzerland) and a Linomat 5 sample applicator were used to perform HPTLC fingerprinting of hexane extracts. As mobile phases, Toluene: Ethyl Acetate (9:1) and Ethyl Acetate-Methanol-Water (100:13.5:10) were used to create the plates. When calculating the Rf values of the bands, it was noted that the extract showed a variety of band counts at various wavelengths. In the second experiment, the methyl extract was run on the Camag-HPTLC system (Switzerland) using a Linomat 5 sample applicator, Toluene: Ethyl acetate (9:1), and Ethyl acetate-methanol-water (100:13.5:10) plates as mobile phases. Both experiments revealed distinct bands at various wavelengths [27]. It is crucial to conduct an HPTLC analysis for any standardization of pharmaceuticals since this technique is recognized to be the first step in analyzing various phytochemical components and secondary metabolites of a plant.

Lead was found to be 0.008 ppm in the present study whereas other studies reported about 0.866 ppm of lead in the plant extract [29]. A non-essential element called lead may enter the human body by eating, inhalation, or cutaneous absorption. Lead is mostly deposited in the bones, even though its concentration in the liver and kidneys can occasionally be rather high. The central nervous system, kidneys, and liver are particularly sensitive to lead's harmful effects [30]. It is a dangerous bodily toxin that builds over time. Lead interferes with several crucial enzymes necessary for the whole process of chemosynthesis, causing metabolic intermediate to build up [31]. The food consumption guideline for lead is 3 mg/week, but the WHO has set a maximum

of 10 ppm for lead concentration in herbal medicines [32].

Cadmium was found to be 0.001 ppm in the present study whereas other studies reported about 0.074 ppm of cadmium in the plant extract [29]. There have been several incidents of food poisoning caused by cadmium, a non-essential trace metal with unknown direct effects in both plants and humans [33]. The arteries of the human kidney are negatively affected by even little amounts of cadmium. It biochemically substitutes zinc and results in elevated blood pressure, renal damage, and other problems. The human body accumulates cadmium, which mostly harms the kidneys and liver [34].

Mercury was found to be 0.002 ppm in the present study whereas other studies reported about 0.062 ppm of mercury in the plant extract [29]. Mercury may be harmful to fetuses and have negative effects on the neurological and renal systems. It can also pass the placental barrier [35], [36]. No sample that was examined had significant mercury concentrations. The acceptable limit of mercury in medications, including those derived from plants, is 0.5 g Hg/g [37].

Arsenic was found to be 0.005 ppm in the present study whereas other studies reported about 0.033 ppm of mercury in the plant extract [29]. It is a poisonous, non-essential element that is frequently found in pesticides, fungicides, and herbicides. Metabolic disease results from high concentrations of arsenic (III) molecules. Along with lung cancer, dermatitis, irritation of the upper respiratory tract, ulceration, and perforation of the nasal septum, cardiovascular and neurological effects are also brought on by it [38].

The study's findings showed that except for S. typhi, the test isolates' growth was reduced by the S. indicus hexane extract. This demonstrates that the extract includes substance(s) that can stop some microorganisms from growing. Flowers and aerial portions of S. indicus showed antibacterial activity when extracted with hexane. 7-hydroxyfrullanolide, a sesquiterpene lactone isolated from S. indicus, exhibited antibacterial action. The presence of alkaloids, tannins, and flavonoids, all of which have been demonstrated to have antibacterial properties—explains the relevance of the antibacterial action. The drug's antimicrobial qualities indicate its utility in conventional medicine. The plant's extracts have reportedly been used in the past to treat boils, heal sores and wounds, and function as an ear drop for boils in the ear. Additionally, it has reportedly been used to treat dysentery and diarrhea. Traditional medicinal practitioners have used the extract to heal sores, bores, and open wounds because of the significant zones of inhibition it displayed against *S. aureus* and *P. aeruginosa*. Boils, sores, and wounds have all been linked to S. aureus and P. aeruginosa. Additionally, it can be observed that growth inhibition of bacteria denotes the use in the management of diarrhea and dysentery. Salmonella typhi may have a method for detoxifying the active components in the extract, which explains why the extract is unable to suppress it. It is

known that some bacteria can transform chemicals that prevent their development into non-toxic molecules. When the antibiotic penicillin is converted to penicillinoic acid by the enzyme penicillinase, *S. aureus* no longer experiences growth inhibition. Against B. subtilis and S. aureus, the antibiotic streptomycin (10 g/disc) had an inhibitory zone that was equivalent to both the flower extract (1.25 mg/disc) and the aerial parts extract (2.5 mg/disc).13 Additional members of the same family of plants displayed comparable antibacterial activity [39]. At 5 mg/disc, a higher inhibitory zone in B. subtilis was found when using hexane flower extract.15 The concentration utilized had a direct correlation with the inhibition zone. The aforementioned findings lead to the conclusion that plant extracts from Sphaeranthus indicus have considerable potential as antibacterial agents against microorganisms and can be utilized to treat infectious disorders brought on by resistant microorganisms. Concern over antibiotic resistance is now widespread. Multiple resistances are becoming more frequent in human pathogenic microorganisms in recent years, partly as a result of the widespread, indiscriminate use of commercial antimicrobial medications used to treat infectious illnesses. This has compelled scientists to look for novel antibacterial compounds from a variety of sources, such as the common tropical medicinal plants. It has a 40-centimeter maximum height. The stem is thin and frequently reddish. The younger portions of the stem are coated with yellowish bristly hairs. The floral and aerial portions of S. indicus exhibited strong antibacterial action against gram-positive pathogens when extracted with hexane. The flower extracts have more potency than the aerial components. Additionally, it demonstrated potent antifungal activity against Candida and other fungi under test. The present study's findings could inspire the creation of organic antibacterial substances.

Skin conditions are treated internally with whole plant powder. To cure skin conditions, leaf, flower, and seed parts are mashed into a paste and administered topically. Whole plant paste is used directly to soothe irritated skin. Making root paste involves blending the root with coconut juice or lemon juice from the sensitive pericarp [40]. The ability of a cream containing an ethanolic extract of *S. indicus*, L. (Asteraceae) aerial parts to promote wound healing in guinea pigs was tested. Once daily for 15 days, the cream was administered in-vivo to the paravertebral region of six excised injured animals. Similar to neomycin, the cream dramatically increased the pace of wound constriction and the duration of epithelialization [41].

Ointments containing varying concentrations of the alcoholic extract of the S. indicus flower head were investigated for their ability to promote improved tissue development while also promoting wound healing. It was discovered that the formulation with 2% (w/w) alcoholic extract was superior to the control and typical formulation. To evaluate the effectiveness and safety of *S. indicus L., crea of Lawsonia inermis L.,* and Plumbi oxide, 45 patients (n

= 30 test and n = 15 control groups) participated in a randomized placebo-controlled single-blind trial. It was discovered that the test medication formulations were successful in treating and reducing the signs and symptoms of cervical erosion with cervicitis [42].

Conclusion:

Since the species' photochemistry and pharmacological functions have been thoroughly investigated, it is necessary to examine and debate all of the scientific information that has been published for this crucial species in terms of ethnomedicine in the form of a comprehensive knowledge product. It is important to research the major flavonoid characteristics in this plant since several studies have shown that flavonoids have many beneficial uses, including acting as anti-inflammatory agents, treatments for skin conditions, and anti-microbial.

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