THE PHYTOCHEMISTRY AND ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS FROM THE GARHWAL HIMALAYA

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Abstract

To find new, safe, and more effective drugs, the present study investigated antioxidant activity, *In vitro* antimicrobial activity, and phytochemical screening of different fractions (petroleum ether, chloroform, ethyl acetate, methanolic, and water) of *Berberis aquifolium* and *Curcuma longa (*fruit and rhizome). The antimicrobial activity of the different fractions of both plants was studied against ten (gram-negative and gram-positive) bacteria. The methanolic fraction of both plants fruit and rhizome parts exhibited potent *in vitro* antimicrobial activity. The methanolic fraction of *Curcuma longa* showed maximum zones of inhibition in these bacteria (24±1mm, 22±1mm, and 21±1mm*) Staphylococcus aureus* > *Staphylococcus epidermidis* > *Escherichia coli*. In phytochemical testing, carbohydrates, glycosides, terpenoids, flavonoids, alkaloids, phenols, resins, and tannins are found in plants.

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Keywords: *Berberis aquifolium,* Haldi, Antimicrobial activity and Phytochemical screening.

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Introduction

Traditional medicines play an important role in health services around the globe. Plants have influenced the development of traditional medical systems, including the popular Ayurvedic, Unani, and Chinese systems. In the Indian, Egyptian, Chinese, Roman, and Greek civilizations, plants served as the cornerstone of the healthcare system and were revered for their alleged divine and supernatural healing abilities. [1, 2]. Medicinal plants represent a rich source of potent drugs. Natural products derived from plants, animals, microorganisms, and minerals are the main treatment methods for human and animal diseases [3]. There are large numbers of medicinal plants which are widely used in the treatment of skin diseases and are also known to possess antimicrobial activity [4]. Plants comprise primary and secondary metabolites, which show and play various therapeutic functions. The primary and secondary metabolites are used as pressure and deterrents. Its miles are referred to as plant herbal products [5].

B. aquifolium (Berberidaceae) is an evergreen shrub growing 1-3 m (3-10 ft) [6] and has dense clusters of yellow flowers in early spring, followed by dark bluish-black berries [7, 8]. *C. longa* (Turmeric, haldi) is a medicinal plant belonging to the ginger family Zingiberaceae which has anti-HIV, antiseptic, anti-inflammatory, antibacterial, antioxidant, antifungal, antiviral, antitumor properties, among others [9]. Curcumin, the main constituent of *C. longa*, is responsible for its beneficial activities. Curcumin displays anticancer, antidiabetic, and anti-inflammatory activities [10]. Cyclooxygenase (C0X-2) has a vital role in the initiation of colon cancer. Curcumin-treated HT-29 colon cancer cells decreased COX-2 expression at different concentrations. Curcumin aids in the prevention of colon cancer and breast cancer cell lines (MCF-7) were assessed through SRB and MTT assays for cytotoxicity and cell viability, respectively which exhibited augmented caspase 3/9 activity and initiation of apoptosis indicating downregulation of miR-21 and the expression of miR-21 in MCF-7 cells by upregulation of PTEN/Akt signaling pathway [11].

Figure 1:-Plants of *Berberis aquifolium* and *Curcuma longa.*

Materials and methods

Plant material

The fresh parts of plants of *B. aquifolium* and *C. longa* were collected from adjoining areas of the 'Himalaya Drug Company Saharanpur' in February 2020. The plant was authenticated by the 'Botanical Survey of India', Dehradun Uttarakhand India.

Chemicals

The following chemicals and reagents were used in the experimental work: sulphuric acid, hydrochloric acid, ethyl alcohol, methanol, sodium hydroxide, Wagner's reagent, Fehling solution A & B, Benedict's reagent, Dragendroff's reagent, Lead acetate, Methanol, Choloform, Acetic Anhydride, Millon's reagent, Mayer's reagent, Sodium bicarbonate, Folin-Ciocalteu reagent (Merck India Limited), and EDTA were used for this study. The solid media and broth used for microbial culture were procured from Hi-Media Pvt. Limited, Bombay, India.

Plant extract preparation

The fruits and rhizomes were dried under shadow for seven days and were crushed into powder by using a mechanical grinder and soxhlet extracted with water, petroleum ether, and methanol utilizing a soxhlet apparatus [12]. Under reduced pressure, the extract evaporated to dryness. In this manner, the acquired extracts were put away in an air tight flask at 4^0C until additional investigation. The prepared plant extract was subjected to different qualitative and the results were carefully evaluated [13].

Detection of chemical compounds by TLC

TLC is a chromatographic method for separating substances. A thin coating of an adsorbent substance, typically silica gel G, cellulose, or aluminum oxide, is placed on a sheet made of aluminum foil, glass, or plastic for TLC [14]. The term 'stationary phase' refers to this adsorbent layer. The sample was placed on the plate and then the solvent or solvent compounds (mobile

phase) were dragged up by capillary action on the plate. TLC plates are made by applying silica gel G to a glass plate and using distilled water as a solvent to activate the plates for an hour at 110^0C. Each extract is used individually and processed through a different solvent solution with a varied level of polarity. These plates can be obtained in a UV light, iodine chamber, and in spraying reagents for various chemical component spots [15].

Successive value

Finely weighed 500gm stiff and air-dried fruits and rhizomes plant materials were exposed to hot progressive persistent extraction in a soxhlet apparatus using various solvents with expansion in methanol, petroleum ether & water as conclusive solvents. The extracts were sieved in each progression and the solvent was taken out by vacuum distillation. The extracts were desiccated in a vacuum and the residues were measured. That contains great chemical compounds that are categorized depending on the nature of the solvent and tie [16].

Radical scavenging assay of DPPH

Using the DPPH assay, the free radical scavenging activity of *B. aquifolium* and *C. longa* was evaluated. Inclination of the example extract of the plant to search for free radicals of DPPH was evaluated using standard techniques along with reasonable alterations. The extract's stock solutions were diluted in methyl alcohol to acquire a 1 mg/ml level. Dilutions of 20, 40, 60, 80 & 100 µg/ml concentrations were made. The dilutions (1ml each) were blended in with 2ml methyl alcohol solution of DPPH (1 mg/ml). Incubation (darkness) for 30 min at room temperature (23^0C) was done and absorbance was observed at 517 nm. All reagents were present in the control specimen aside from the concentrate and the rate hindrance was plotted in a concentration plot, using a nonlinear regression algorithm [17, 18].

$$\text{Inhibition (\%)} = \frac{\{(\text{Absorbance of control}) - (\text{Sample absorbance}) \times 100\}}{(\text{Absorbance of control})}$$

Phytochemical analysis

Standard procedures were used to analyze the qualitative phytochemical composition of plant samples. The above-mentioned extracts are subsequently put through qualitative tests to identify the various chemical components of plants. Additionally, hydro-distillation is used to identify the presence of volatile oil in 50 grams of fresh or moisture-dried material from the plant. The plant material could go through preliminary phytochemical screening to find different plant elements along these lines [19, 20].

Antimicrobial activity

The antimicrobial activity testing was performed by relating the diameter of zones of inhibition (in mm), which indicates the effectiveness of an antimicrobial agent [21]. Different extracts of *B. aquifolium* and *C. longa* were observed for their antimicrobial properties against different pathogens, such as *Staphylococcus aureus* (MTCC-6538P), *Escherichia coli* (MTCC-8739), and *Candida albicans* (MTCC-18804). Its activity was also compared with standards, such as Erythromycin (10 mg/ml). It is believed that the antimicrobial property might be due to the presence of strong flavonoids in the extract and the weak antioxidant nature of the extract, which in turn increases the shelf life of the product from photodegradation and oxidative degradation. The antimicrobial property of the extract might be due to the high percentage content of flavonoids,

which makes the preparation highly effective against the studied microorganisms. Additionally, the antioxidant property that protects epidermal cells from UVA-induced damage is mainly due to the presence of orthophosphoric acid in the gel, which protects the skin from conditions of extreme pH modification [22].

Statistical analysis

The data are expressed as the mean ± SEM analyzed by one-way analysis of variance (ANOVA) and Tukey's t-test was used as the test of significance. P value<0.05 was considered as the minimum level of significance. All statistical tests were carried out using SPSS statistical software [23].

RESULTS AND DISCUSSION

Table 1. Organoleptic characteristics and extractive value of ***B. aquifolium*** and ***C. longa*****.**

S. No	Plants	Color	Odor	Methanol extract (%)	Water extract (%)	Ash Value (%)	Acid soluble extract (%)	Foreign matter (%)
1.	*Berberis aquifolium*	Bright and deep color	Reminiscent of roses	5.00	11.0	8.60	0.50	1.70
2.	*Curcuma longa*	Deep yellowish-brown color	Mild aromatic	5.50	10.5	9.15	0.80	1.35

Table 2.Observations from TLC studies of *B. aquifolium* and *C. longa*.

Type of extract	"Mobile" phase	Number of spot	"Rf" value
Petroleum Ether (Leaves)	Ethyl acetate and hexane (2:8)	5	1) 1.11 2) 1.33 3) 1.81 4) 2.41 5) 4.61
Petroleum Ether (Root)	Benzene and acetic acid (8:2)	4	1) 0.23 2) 0.63 3)0.69 4) 0.78
Methanol(Leaves)	Ethyl acetate andn- Hexane	1	1) 3.52

Table 3. Antioxidant activities (mm AAE/100G FW) of ***B. aquifolium*** and ***C. longa***.

Methanol plant extract	ABTS Assay	FRAB Assay	DPPH Assay
Berberis aquifolium	12.46 ± 0.10	12.13 ± 0.03	40.51 ± 0.15
Curcuma longa	13.27 ± 0.03	12.69 ± 0.18	39.02 ± 0.58

Table 4. Antibacterial activity of ten bacterial strains against *B. aquifolium,* and *C. longa*.

Bacterial Name		Erythromycin	*BA* Methanol Extract	*CL* Methanol Extract
Genus /Species/Subspe.	MTCC(Code)	10Mg/ml	50 mg/ml	50 mg/ml
Bacillus cereus	1272	12	16	17
Escherichia coli	729	14	16	17
Enterobacter gergoviae	621	13	17	18
Klebsiella pneumonia	432	11	16	17
Salmonella entericatyphim	98	10	17	19
Shigella flexneri	1457	10	17	18
Staphyloccus aureus	902	11	22	24
Staphyloccus epidermidis	435	10	21	22
Streptococcus pyogenes	1925	12	19	20
Escherichia coli	443	13	19	21

Table 5. Antifungal activity of three fungal strains against ***B. aquifolium and C. longa***.

Fungal Name		Ketoconazole	*BA* Methanol Extract	*CL* Methanol Extract
Genus /Species/Subspe.	MTCC(Code)	10mg/ml	50 mg/ml	50 mg/ml
Candida albicans	3017	10	13	14
Aspergillus flavus	2798	8	12	13
Aspergillus parasiticus	2796	9	12	13

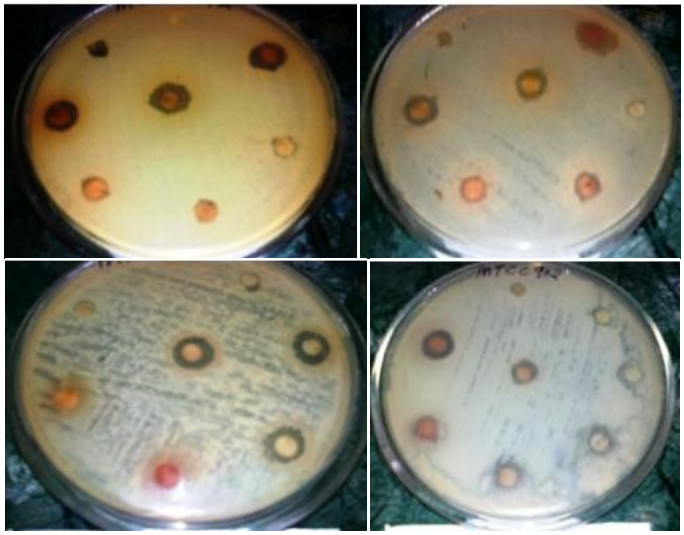
Note: - Disc size, 5 mm, Inhibitory zone size ±1 mm, and mm means (millimeters) and – Indicate (NIZ) No inhibitory zone.

Table 6. Phytochemical screening of ***B. aquifolium*** and ***C. longa*** plants.

Test	*BA* Extract	*CL* Extract
Carbohydrates/ glycosides (1) Molish test (2) Fehling test (3) Benedict test	**(-)** **(+)** **(+)**	**(-)** **(-)** **(-)**
Alkaloid (1) Mayer's test (2) Dragondroff test	 **(-)** **(-)**	 **(+)** **(+)**

Flavonoids (1) Shinoda/pew (2) Ammonia	 **(+)** **(+)**	 **(+)** **(-)**
Saponins	**(+)**	**(+)**
Tannins (1) Pyrogoll& catechol (2) Gallic acid	 **(+)** **(+)**	 **(+)** **(+)**
Unsaturated sterol/triterpenes (1) Liebermann Burchard test (2) Salkowiskis test	 **(+)** **(+)**	 **(+)** **(+)**
Phenolics compound (1) Ferric chloride	**(+)**	**(+)**
Protein and amino acid (1) Xanthoprotien	**(+)**	**(+)**

Figure 1:-Antibacterial and antifungal activity of ***B. aquifolium,*** and ***C. longa*** extracts against disc diffusion methods**.**



Plants are considered to be a vital source of potentially useful constituents for the development of new therapeutic agents, as most of them are safe with less or no side effect(s). The antioxidant activity of *B. aquifolium*, and *C. longa* different extracts (petroleum ether, methanol, and water) was estimated as far as their effective IC-50 concentration relates to the concentration of the sample which decreased the initial DPPH absorbance of 50%. Detection of a photochemical compound through TLC. Petroleum ether and methanolic extracts (fruits and rhizomes) phytochemical screening leads to the identification of alkaloids, phenolic compounds, tannins, proteins, terpenoids, and glycosides.

CONCLUSION

The present research was done to study the antioxidant activity, and phytochemical screening of *B. aquifolium,* and *C. longa* to search for new, safer, and more effective drugs. In this research, the antioxidant activity, and phytochemical screening of distinctive extracts of the fruits and rhizomes of *B. aquifolium,* and *C. longa* by using standard methods were evaluated. The TLC result of petroleum ether extracts gives the maximum spots in the mobile phase (ethyl acetate and n-hexane) solvent. It can be concluded that the different extracts of *B. aquifolium* and *C. longa* possess potent antimicrobial activity. Now our research will be directed at developing a broad-spectrum antimicrobial polyherbal formulation from this plant. Even at low concentrations. This analysis revealed that the fruits and rhizomes contained higher values of antibacterial & antifungal activity as compared to other plants.

Disclosure of interest

The authors declare that they have no competing interests.

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