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Comparative analysis of total phenolic content and antimicrobial activity of some plants of Garo hills, Meghalaya

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Abstract
Eethanolic and aqueous extracts of some plants commonly found in Garo hills of Meghalaya and known for their medicinal value were analysed for total phenolic content and antimicrobial activity. Total content of phenols was quantitatively estimated from extracts of lemon grass, tamarind, Ber, starfruit and Beetle nut. Antimicrobial activity and yield of these plant extracts was also determined. Further, ethanolic extracts have revealed better total phenolic content and antimicrobial activity against test pathogens in comparison to aqueous extracts. Among the plant extracts investigated Beetle nut exhibited highest content of phenols and also best antimicrobial properties. The results show that use of the natural antimicrobial inherent properties occurring in plants extracts is a viable option for the textile finishing industry for functional textile applications. The plant extracts showed high antimicrobial activity which indicates that these extracts may be useful natural remedies for antimicrobial textile finishes for treatment of various types of skin/wound infections and medical textile applications.

Keywords: Functional finishes, total phenolic content, antimicrobial activity, plant extracts

Introduction
Plants are a sustainable source of medicinal products especially in traditional medical practices. Plants contain active substances such as alkaloids, tannins etc., produced during their secondary metabolism which serve as a potential reservoir of medicinal products (Hussein and El-Anssary, 2018) [1]. Traditionally, plant extracts have been used for the treatment of pain, inflammation and other musculoskeletal disorders. Medicinal properties of plant extracts may be attributed to secondary metabolites found only in plants belonging to certain species and family, which have been shown to provide protection against pests, animals or UV radiation. Some of the secondary metabolites may be pharmacologically active in humans and useful as medicines. Major secondary metabolites found in plants are phenolics, alkaloids and terpenoids (Tungmunnithum *et al*., 2018) [2].
Garo hills of Meghalaya are home to several plants, which are being used by local people for medicinal purposes (Sangma and Sahoo, 2017, Sharma *et al*., 2014) [3, 4]. Various plant species contain phytochemicals which may be effective for the treatment of certain chronic diseases because they possess antioxidant, antibacterial, antiviral, anti-carcinogenic activities (Zhang *et al*., 2015, Ramona *et al*., 2017) [5, 6]. Despite their significant potential, scarce efforts have been made to explore the medicinal use of plants found in Meghalaya. Therefore, exploration and judicious exploitation of these medicinal plants is required, which will facilitate research in pharmaceutical industry to identify the bioactive components that possess herbal properties. Hence, the present study aimed at determining the amount of total phenolic content as well as antimicrobial activity present in some plants *viz*., lemon grass, tamarind, Ber, starfruit and Beetle nut commonly found in west Garo hills, Meghalaya. Present investigation is an effort to explore the role of native herbs or plants having medicinal properties as herbal agents in herbal clothing finishes.

Material and Methods

Medicinal Plants

Medicinal plants selected for this study are commonly found in Garo hills of Meghalaya and are well known for their medicinal properties. Five plant samples were collected from West Garo hills District of Meghalaya (Table-1).

Table 1: Medicinal plants selected for the study

Sl. No.	Local name	Scientific name	Part used
1	Lemon grass	*Cymbopogon ctratus*	Leaves
2	Beetle nut	Arecanut catechu	Seeds
3	Ber	*Ziziphus jujube*	Leaves
4	Tamarind	*Tamarindus indica*	Leaves
5	Starfruit	*Averroha carambola* L.	Leaves

Preparation of Extracts

The matured leaves of lemon grass, Ber, Tamarind, Starfruit plants and ripened Beetle nut seeds were collected, cleaned with distilled water and dried at 40°C to remove the traces of moisture. Plant material was then crushed using mechanical grinder and sieved to obtain fine powder.

Biochemical extraction

Plant extracts were prepared using 70% ethanol (ethanolic extract) and distilled water (aqueous extract) as solvents for analysis of phyto-constituents. Briefly, 2.0 gram of dry powder was mixed separately in 25ml of each solvent (70% ethanol and distilled water) and incubated for 24 hours at room temperature, later centrifuged at 5000 rpm (REMI C-24 Plus refrigerated centrifuge) and the supernatants were separated. Residues were extracted with 25 ml of the respective solvent and the process was repeated. The supernatants obtained were pooled and the extracts obtained were measured and filtered using Whatman filter paper No. 40 (125 mm). Extracts were stored at 8 ºC for further analysis within 7 days (Vastrad *et al.*, 2015) [7].

Fig 1: Aqueous herbal extract

Fig 2: Ethanol herbal extract

Estimation of total phenol content (TPC)

Total Phenolic Content (TPC) in the extracts was determined by Folin-Ciocalteu assay method (Singleton and Rossi, 1965) with little modification using gallic acid as the reference standard. Briefly, all the extracts were diluted to appropriate volumes and were mixed with 2 ml of 10% Na_2CO_3 solution, incubated at room temperature for 3 min, later 100 µl of Folin-Ciocalteu reagent was added to the mixture. The resulting solution was incubated for 90 min at room temperature under dark; the absorbance was measured at 765 nm using the UV-Vis Spectrophotometer (BioMate 3S UV-Visible Spectrophotometer). The TPC was expressed as Gallic acid equivalent (GAE) in milligrams per gram of dry powder.

Antimicrobial activity of plant extracts

Bioassay was carried out to assess the antibacterial activity of the plant extracts by Well Diffusion Method. The bacterial species *viz., Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739) were used for the study. Nutrient agar and nutrient broth media were prepared separately in distilled water and autoclaved at 121 ºC for 15 minutes at a pressure of 15 lbs. A loopful of bacterial cultures (*S. aureus* and *E. coli*) were mixed separately in the nutrient broth and kept under shaking condition for 24 hours. The nutrient broth containing bacterial inoculum was mixed with sterile nutrient broth and was uniformly spread on sterile Petri plates and allowed to solidify. Later, four wells were created using a cork borer (5 mm diameter). The plant extracts were added to each of the respective 3 wells, one well of being the control sample. Seventy per cent ethanol and distilled water were used as control. Each sample was used in triplicates. The Petri plates were incubated for 18-24 hrs at 37 ºC and observed for bacterial growth. Zone of inhibition of the bacterial growth was measured in millimeters (W.H.O., 1991) [9].

Statistical analysis: Statistical analysis was done as described by Snedecor and Cochran and results were expressed in Mean ± SD.

Results & Discussion

Total yield of the extracts obtained per 50ml of solvent is given in Table-2. In general, ethanolic extraction gave better yield than distilled water. Maximum yield was recorded for Beetlenut (*Arecanut catechu)* followed by Lemon grass (*Cymbopogon ctratus)* for both the solvents.

Table 2: Yield of Plant Extracts

Sl. No.	Local name	Scientific name	Part used	Yield of extracts (ml/50ml)	
				70% Ethanol	Aqueous
1	Lemon grass	*Cymbopogon citratus*	Leaves	25.0	21.0
2.	Beetle nut	Arecanut catechu	Seed/Nut	35.6	32.6
3.	Ber	*Ziziphus jujube*	Leaves	19.4	16.0
4.	Tamarind	*Tamarindus indica*	Leaves	23.6	21.6
5.	Starfruit	*Averroha carambola* L.	Leaves	21.4	19.6

Bioactive compound i.e. total phenolic content of the plant sources were determined by using the Folin- Ciocalteu reagent in ethanol and aqueous extracts. Plant phenols represent one of the major groups of bioactive compounds acting as primary antioxidants or free radical terminators.

Significant variation in the total phenolic content of the five plant extracts ranging from 13.81±0.36 GAE mg/g in Lemon grass aqueous extract to 163.07 ± 0.16 GAE mg/g in Beetle nut ethanolic extract was evident in present study (Table-3).

Table 3: Total Phenolic Content (TPC) of plant extracts

Sl. No.	Local name	Scientific Name	Part used	Total Phenolic Content GAE(mg/g dried source)	
				70% ethanol	Aqueous
1	Lemon grass	*Cymbopogon ctratus*	Leaves	26.54±0.60	13.81±0.36
2	Beetle nut	Arecanut catechu	Seed/Nut	163.07± 0.16	112.14±0.13
3	Ber	*Ziziphus jujube*	Leaves	43.78±0.24	22.33±0.64
4	Tamarind	*Tamarindus indica*	Leaves	47.72 ± 1.60	38.20 ± 1.24
5	Starfruit	*Averroha carambola L.*	Leaves	65.16 ± 1.53	33.39±0.38

The results showed that ethanolic extracts had higher values of TPC than the aqueous extracts. The highest TPC was recorded in Beetlenut (*Arecanut catechu*) followed by starfruit (*Averroha carambola* L.,) tamarind (*Tamarindus indica*), Ber (*Ziziphus jujube)* and lemon grass (*Cymbopogon citratus* (Table-3).

Agar gel well diffusion method was used to asses antimicrobial potential of plant extracts against standard test cultures *viz*. *Staphylococcus aureus* (gram positive) and *E. coli* (gram negative) organisms. It was observed that, ethanol extracts of lemon grass showed antibacterial activity against both *S. aureus* (3.5±0.42mm) and *E. coli* (7.5±0.62mm), respectively, whereas distilled water extract of lemon grass showed no inhibition zone against both the organism. Ethanol (7 ± 0.82mm) and distilled water (6 ± 0.68mm) extracts of *starfruit showed* good antibacterial activity against *S. aureus* but antibacterial activity against *E. coli* was absent in starfruit extracts.

Ethanol extract of Beetlnut was found to exhibit maximum zone of inhibition (10±0.71mm) against *Staphylococcus aureus* followed by Ber leaves (9 ± 0.84mm), starfruit leaves (7±0.82 mm), tamarind (6±0.45mm) and lemon grass (3.5±0.42). Among the bacterial species, comparatively higher inhibition zone was observed against Gram positive (*Staphylococcus aureus*) bacteria than Gram negative (*Escherichia coli*) bacteria.

Table 4: Antimicrobial activity of herbal extracts

Sl. No	Plant extracts	Zone of inhibition (mm)			
		S. aureus		*E. coli*	
		Distilled Water	70% Ethanol	Distilled water	70% ethanol
1.	Lemon grass (*Cymbopogon citratus*)	Nil	3.5±0.42	Nil	7.5±0.62
2.	Beetle nut (Arecanut catechu)	4±0.68	10±0.71	5±0.82	8.5 ±0.82
3.	Ber (*Ziziphus mauritiana)*	5 ± 0.32	9 ± 0.84	Nil	8 ± 0.54
4.	Tamarind *(Tamarindus indica)*	Nil	6±0.45	Nil	7 ± 0.86
5.	Starfruit *(Averroha carambola.* L.)	6 ± 0.68	7 ± 0.82	Nil	Nil

The results obtained in the present study suggest that plant extracts derived from lemon grass, tamarind, Ber, starfruit and Beetle nut have moderate to potent antimicrobial activity (Table-4). Polyphenolic compounds have some known properties which include free radical scavenging, antimicrobial, antiallergic activity and anti-inflammatory action etc. (Dugo *et al*., 2017, Ferreres *et al*., 2017, shah *et al*., 2011) [11, 12, 13]. Experimental plant extracts contained appreciable amount of total phenol, which showed that the antimicrobial activity of the plant extracts is influenced by the total phenolic compounds (Elnaggar *et al*., 2019) [14]. These differences in the antioxidant activities may be due to their differences in phenolic contents and compositions and also due to other non-phenolic antioxidants present in the samples (Tungmunnithum *et al*., 2018, Elnaggar *et al*., 2019) [2, 14].

A study using different concentrations of lemon grass oil showed activity against *S. aureus*, *E. Coli* and other bacteria (Naik *et al*., 2010) [15]. Our findings are comparable with activity of 5% essential oil against *E. Coli*, comparatively smaller inhibition zone against *S. aureus* may be due to difference in sample preparation. Furthermore, smaller zone do not necessarily indicate lesser antimicrobial potential (W. H. O., 1991) [9]. Ethanolic and aquous extracts of *Arecanut catechu* has earlier been shown to possess antibacterial activity against *Staphylococcus aureus* (Rahman *et al*., 2014) [16]. However, in our study both extracts exhibited activity against *Staphylococcus aureus* and E. *Coli*, this differenc in activity may be attributed to different extraction procedure, type of arecanut and different strains of test organisms. Fruits, seeds and leaves of Ber are believed to possess antimicrobial activity (Pandey and Poonia, 2018) [17]. Methanolic extract has been shown to possess antibacterial activity against *Staphylococcus aureus* (Emad *et al*., 2016) [18]. Flavonoids and polyphenols found in *Tamarindus indica* leaves have been recorded as antimicrobial agents (Doughari, 2006) [19]. Abdallah and Ali (2018) [20] also reported antibacterial activity of tamarind leaves and fruit extract against clinical isolates of *E. coli* and *Shigella* sp. Starfruit extracts in various concentrations were shown to inhibit the growth of *Staphylococcus aureus* and *Klebsiella* spp. (Chang *et al*., 2000) [21]. Extracts were also effective against human pathogens like *Escherichia coli, Shigella boydii, Salmonella* Typhi and *Staphylococcus aureus, Candida albicans* and *Candida krusei* (Ma and Wai, 2017, Silva *et al*., 2020) [23].

In conclusion study demonstrates that ber leaves. Lemon grass, Tamarind and starfruit leaves extracts have antimicrobial properties and hence could be exploited as antimicrobial finishes for medical textile and other similar applications for human use.

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