Evaluation of *Garcinia mangostana*, *Lantana camara* and *Piper betel* for Pharmaceutical Applications

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Received: 22nd February, 2023; Accepted: 30th March, 2023; Published online: 24th April, 2023
https://doi.org/10.33745/ijzi.2023.v09i01.082

Abstract: Plant extracts are known to have a number of beneficial properties and one among them is the antimicrobial property. From various studies it has been concluded that *Moringa oleifera*, *Piper betel*, *Garcinia mangostana*, *Lantana camara* and *Premna serratifolia* possess significant antimicrobial compounds. This study was aimed at exploiting this property for the production of skin friendly plant based sanitizers. Plant extract preparation was done using absolute ethanol for the five plant species. The yield obtained was dissolved in calculated proportion of ethanol. Similar procedure was followed for the preparation of sanitizers, where absolute ethanol was replaced with 98% glycerol and 70% isopropyl alcohol (IPA). Essential Oils were extracted from *Cymbopogon citratus*, *Jasminum officinale*, *Hedychium coronarium* and *Pimenta dioica* using simple distillation and soxhlet extraction for the purpose of serving as an aromatic agent. The antioxidant property of the plant extracts was determined by using DPPH assay and the antimicrobial property of the plant extract, essential oil and prepared sanitizer was tested using agar well diffusion technique on Muller-Hinton agar (MH agar) against MTCC strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Garcinia mangostana* exhibited the maximum radical scavenging activity (89.58%). Among the five ethanolic extracts, *Lantana camara* showed the highest inhibitory activity against the three test organisms while *Moringa oleifera* gave the least inhibitory activity. The sanitizer prepared from *Garcinia mangostana* was found to possess the maximum sterilizing activity. Out of the five plant species chosen three (*Garcinia mangostana*, *Lantana camara*, *Piper betel*) were found to have sterilizing activity and can be used commercially for the production of skin friendly sanitizers.

Keywords: *Moringa oleifera*, *Piper betel*, *Garcinia mangostana*, *Lantana camara*, *Premna serratifolia*, Ethanolic extract, Antimicrobial activity, Agar well diffusion technique, Sanitizer, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

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Introduction

Maintaining hand hygiene by cleansing of your hand with soap and water, usage of antiseptic hand scrubs such as alcohol-based sanitizers or surgical antiseptics is recommended by authorities all over the world (Toney-Butler et al., 2022). As a result of publicity and convenience of its use, there has been multiple reports of overuse of these alcohol-based hand sanitizers (ABHS), leading to increasing incidence of skin disorders like irritant contact dermatitis (ICD), allergic contact dermatitis (ACD), dryness of skin, hand eczema, and many more. (Jindal et al., 2020)

Plant extracts are known to have numerous bioactive molecules with potential applications as therapeutic agents, cosmetics, functional food additives, and they are also known to possess antimicrobial properties. This property of plant extract, of inhibiting microbial growth is exploited to produce plant-based sanitizers, making these a safe and friendly alternative to the conventional alcohol-based hand sanitizers. (Patankar and Chandak, 2018)

Materials and Methods

Collection of materials:

The leaves of the plants, *Garcinia mangostana* (mangosteen leaves), *Lantana camara*, *Moringa oleifera* (drumstick leaves), *Piper betel* (betel leaves) and *Premna serratifolia* (Headache tree) were collected from places in and around Mangalore, India. The leaves were washed with distilled water and then dried in a hot air oven maintained at 45°C. The dried leaves were powdered and stored in air-tight containers at room temperature.

Preparation of extract:

The ethanolic extract was prepared by mixing the powder and absolute ethanol in 1:4 (w/v) ratio (Mostafa et al., 2018).

Determining the antioxidant activity using DPPH radical scavenging method:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of *Garcinia mangostana*, *Lantana camara*, *Moringa oleifera*, *Piper betel* and *Premna serratifolia* was determined by using the method of Kartal et al. (2007).

The ethanolic extracts prepared were diluted in the ratio 1:150 (1 ml of extract in 150 ml of ethanol). 1 ml of the five diluted plant extracts was added to 5 ml of 0.004% methanol solution of DPPH. The solutions were incubated in dark condition for 30 min at room temperature and the absorbance was read at 517 nm using methanol as blank.

Extraction of essential oils:

The essential oils from the leaves of *Cymbopogon citratus* (Lemongrass) and *Pimenta dioica* (All Spices), and the flowers of *Hedychium coronarium* (Sugandhi) and *Jasminum officinale* (Jasmine) were extracted using simple distillation. The essential oil from *Jasminum officinale* was also extracted using soxhlet and hexane as the solvent. *Cymbopogon citratus* and *Pimenta dioica* were washed and dried in a hot air oven maintained at 45°C. The dried leaves were powdered and stored in an airtight container at room temperature. The flowers of *Hedychium coronarium* and *Jasminum officinale* were collected on the day extraction was performed.

Batch distillation for the extraction of essential oils:

50 g of the plant materials were extracted using 400 ml distilled water. Temperature was set to 100 °C and left to run for 2 to 3 h. The condensate collected was stored in a glass bottle at 19 °C.

Soxhlet extraction for essential oil from *Jasminum officinale*:
30 g of flowers were subjected to extraction using hexane as the solvent. The temperature was set to 55°C. The set up was allowed to run for 3 to 4 cycles. The essential oil that was collected in the boiling flask was concentrated using the simple batch distillation process.

**Preparation of Sanitizer:**

The sanitizers from the extracts of *Moringa oleifera*, *Piper betel*, *Garcinia mangostana*, *Lantana camara* and *Premna serratifolia* were prepared as per the formulation proposed by Patankar and Chandak (2018). The sanitizer was prepared by the addition of 70% isopropyl alcohol, glycerol and the extracted essential oil to the dried extracts obtained in the ratio 1:18:2:4 (Dried extract: 70%IPA: Glycerol: Essential oils).

To the sanitizer prepared from dried *Garcinia mangostana* extract the essential oil from *Cymbopogon citratus* was added, for *Lantana camara* the oil added was from *Pimenta dioica*, extract from *Piper betel* was mixed with the essential oil from *Hedychium coronarium* and finally for *Moringa oleifera*, lemon oil (*Cymbopogon citratus*) was used.

**Antimicrobial activity:**

(a) **Ethanolic extract:**

The agar well diffusion technique was applied to test the antimicrobial activity of the ethanolic extract.

(b) **Essential Oil**

To determine the antimicrobial properties of the essential oils, the following procedure was performed: the wells punched were loaded with the five essential oils, i.e., four extracted from simple distillation and one from soxhlet extraction, and incubated for the same time duration. The tests were performed in duplicates.

**Results and Discussion**

**Extract yield obtained:**

The extract yields obtained from different plant leaf powder using ethanol are given in the Table 1. The highest yield was obtained for *Lantana camara* (5.08%) followed by *Moringa oleifera* (4.09%) and the lowest percentage of yield was obtained from *Piper betel* (2.7%).

The percentage of radical scavenging activity is illustrated in Figure 1. It is evident that the scavenging activity of *Garcinia mangostana* and *Piper betel* were found to be almost equal and possess immense scavenging activity when compared with the rest. This finding derives support from the work of Pin *et al.* (2010) where they obtained 76.87% inhibition for *Piper betel* for a concentration of 0.5 mg/ml and the from findings of Susanti (2019) as they noticed 44.9% scavenging activity for *Garcinia mangostana* (peel).

**Antimicrobial sensitivity test:**

(a) **Ethanolic Extract Preparation:**

Antimicrobial sensitivity test performed by following agar well diffusion technique using the ethanolic plant extract. 200 µl of the extracts (concentration 20 g/l) was loaded into the wells punched on Muller-Hinton agar and inhibition zone sizes (cm) was obtained after 24 h incubation (Fig. 2).

From Figure 2 it can be inferred that the highest inhibiting activity was shown by the ethanolic extract of *Lantana camara* for *E. coli* (3 cm). This finding is backed up from the study of Saraf *et al.* (2011) as they obtained an intermediate zone size for *E. coli* (zone size 1.82 cm/18.2 mm), and Barreto *et al.* (2010) who have reported that the minimum inhibitory concentration for *E. coli* was 128 µg/ml for ATCC strain. The inhibition property of *Lantana camara* against *P. aeruginosa* and *Staphylococcus aureus* was found to be around 2.8 cm (28 mm) and 2.7 cm (27 mm), respectively. Among the five ethanolic extracts the inhibiting property of *Lantana camara* was the best, and the second highest inhibiting property was found to be that of *Piper betel*. 

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Table 1: Yields obtained for various plant leaf extracts in gram and percentage

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Yield (g) mean ± standard deviation</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Piper betel</em></td>
<td>0.138167 ± 0.08209</td>
<td>2.7%</td>
</tr>
<tr>
<td><em>Moringa oleifera</em></td>
<td>0.204667 ± 0.090684</td>
<td>4.09%</td>
</tr>
<tr>
<td><em>Lantana camara</em></td>
<td>0.254067 ± 0.092794</td>
<td>5.08%</td>
</tr>
<tr>
<td><em>Garcinia mangostana</em></td>
<td>0.187867 ± 0.02804</td>
<td>3.75%</td>
</tr>
<tr>
<td><em>Premna serratifolia</em></td>
<td>0.202267 ± 0.149436</td>
<td>4.04%</td>
</tr>
</tbody>
</table>

Fig. 1: DPPH assay of the five plant leaf extracts. Concentration of the extracts being 133 µg/ml. The readings were taken at 517 nm using a UV-Visible spectrophotometer.

Fig. 2: The inhibition zone size obtained on the application of the different plant leaf ethanolic extracts to *E. coli*, *P. aeruginosa* and *Staphylococcus aureus*. 
Table 2: Percentage of radical scavenging activity of the five plant extracts

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Percentage of radical scavenging activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Piper betel</em></td>
<td>89.31±8.556</td>
</tr>
<tr>
<td><em>Moringa oleifera</em></td>
<td>27.56±11.960</td>
</tr>
<tr>
<td><em>Lantana camara</em></td>
<td>64.25±38.727</td>
</tr>
<tr>
<td><em>Garcinia mangostana</em></td>
<td>89.58±4.990</td>
</tr>
<tr>
<td><em>Premna serratifolia</em></td>
<td>73.65±8.934</td>
</tr>
</tbody>
</table>

(b) **Essential Oils:**
The essential oils extracted gave no inhibition zones mostly due to dilution of the extract on the usage of distilled water as the solvent.

(c) **Plant based sanitizers:**
The inhibition zone (in cm) obtained by agar well diffusion technique on MH agar upon loading 200 µl of the sanitizer in which the concentration of the extracts present is 42 µg/ml (Table 2).

The maximum inhibitory activity when tested against *E. coli*, *Staphylococcus aureus* and *P. aeruginosa* was obtained for the sanitizer prepared from *Garcinia mangostana*, with *E. coli* being most susceptible, followed by *Staphylococcus aureus* and *P. aeruginosa* was comparatively less susceptible to the preparation. The second most effective sanitizer preparation was that of *Piper betel* (Fig. 3). The sanitizer prepared from *Moringa oleifera* had absolutely no inhibitory effect. This could be because of the extract’s incompatibility with isopropyl alcohol since it exhibits some antibacterial activity when prepared using ethanol (Fig. 2). *Lantana camara* which gave the best antibacterial activity in ethanol (Fig. 2) possessed lesser antibacterial activity in comparison to *Garcinia mangostana* and *Piper betel* (Fig. 3). The reason might be the same as that in the case of *Moringa oleifera*, that the extract of *Lantana camara* is ineffectual when dissolved in isopropyl alcohol. *Premna serratifolia*, although did exhibit an inhibition zone, the inhibitory activity of the sanitizer prepared was mostly on the resistant side.

(d) **Sterilizing activity of solvents:**
As observed from Figure 4, glycerol gave
absolutely no inhibition zone, thus, indicating that it does not possess any antibacterial activity. 70% IPA and 70% ethanol gave inhibition zones, but the diameters of the zones formed were less than or equal to 2 cm. The inhibition zones formed on the application of ethanolic extracts and sanitizers, gave zone diameters greater than 2 cm, except in the case of *Moringa oleifera* where the zone size obtained for ethanolic extract against *Staphylococcus aureus* was 1.75 cm (Fig. 2) and that obtained for sanitizers was zero in all three cases (Fig. 3). This observation indicates that while the solvents do have some effect on the antibacterial activity of the ethanolic extracts and sanitizers, by and large it is the bioactive compounds present in the extracts that give the formulation the desired antibacterial or sterilizing activity.

**Conclusion**

The secondary metabolites contained in plants i.e., the Phytochemicals, have long been accepted in Pharmacological studies serving as antioxidants and antiseptics. As per the study conducted using *Garcinia mangostana* (mangosteen leaves), *Lantana camara* and *Piper betel* (betel leaves) showed exorbitant Radical Scavenging Activity as well as an acceptable antimicrobial activity while *Premna serratifolia* (Headache tree), *Moringa oleifera* (drumstick leaves) did not exhibit the required inhibitory range in both cases. High percentage due to quercetin, epicatechin, rutin, catechin, and cyanidin-3-sophoroside and the antibacterial activity is mainly due to α-mangostin Eugenol, hydroxychavicol, and gallic acid strongly contribute to the antioxidant activity in *Piper betel* and the antibacterial activity is due to phytochemicals, such as alkaloids, terpenes, anthraquinones, flavonoids, tannins, saponins and steroids.

Out of the five only three of the sanitizer formulations from *Garcinia mangostana*, *Lantana camara* and *Piper betel* can be further improved on and introduced into the market as a potential alternative to the existing alcohol-based sanitizers and hand-scrubs. These plant species can be exploited to produce alcohol-free, skin friendly plant-based sanitizers.

**References**


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