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Analysis of phytochemical, antimicrobial and free radical scavenging activity of bottle gourd (*Lagenaria siceraria*) & neem (*Azadirachta indica*) medicinal plants of Hazaribag

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Abstract

Bottle gourd (*Lagenaria siceraria*) belongs to the gourd family of cucurbita has been used traditionally in medications, Neem (*Azadirachta indica*) commonly known as Indian lilac belongs to Meliaceae family has been used by ayurvedic practitioners for anthelmintic, antifungal, sedative etc. Since the two plants harbour important secondary metabolites, the phytochemical properties of the plants were compared and different solvents systems polar as well as non polar was used for the extract preparation, which yielded similar as well as different findings. Upon the presence of secondary metabolites, antimicrobial and free radical scavenging attributes of the two plants were also studied. A reflection of plant as a source of important primary and secondary metabolites was analysed. The presence of flavonoids, saponins, terpenoids, steroids and few other secondary metabolites were common findings which fluctuated according to the solvent used and also with the plant part that was used for sample preparation i.e. Leaves, stem, fruit etc.

Keywords: *Azadirachta indica*, *Lagenaria siceraria*, phytochemicals, antimicrobial, free radical

Introduction

The vegetable Gourd, belonging to the family *Cucurbitaceae* (Warrier *et al*, 1995). The Bottle gourd (*Lagenaria siceraria*) is popularly known as Lauki, Ghia or Dudhi in India. Its consumption is advocated by traditional healers for controlling diabetes mellitus, hypertension, liver diseases, weight loss and other associated benefits. *Lagenaria siceraria* (white flower gourd) is warm season fruit vegetable which is grown throughout India and is available throughout the year. The centre for origin has been located at the coastal area of Malabar (North Kerela) and humid forest of Dehradun. The *Lagenaria siceraria* fruits are traditional used for its cardio protective, cardi tonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons, scorpion strings, and alternative purgative and cooling effects (Kiritkar *et al*, 2001). It cures pain, ulcers and fever and used for pectoral cough, asthma and other bronchial disorders especially syrup prepared from the tender fruit (Sivarajan and Balchandra, 1996). As well as *Azadirachta indica* been introduced and established throughout the tropics and subtropics for its highly valued hardiness, it's almost year-round shade, and its multiple wood and non-wood products. Although the exact origin of *Azadirachta indica* is unknown, it is thought to have originated in the Myanmar region and to be distributed naturally throughout the Indian subcontinent. *Azadirachta indica* has served in the pharmacological activities and medical applications by the use of its various parts, the biological activities of *Azadirachta indica* has reported with the crude extracts and their different fractions from leaf, bark, root, seed, and oil. Aqueous extract of *Azadirachta indica* leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose induced hyperglycemia (Nadkarni 1996). Extracts of *Azadirachta indica* leaves, neem oil and seed kernels are effective against certain human fungi, including *Trichopython*, *Epidermopython*, *Microsporum* and *Candida* (Fard *et al*, 2008). The aim of this research was to find out the different aspects of phytoconstituents, their activities in further pharmacological evaluation that can help in findings of antimicrobial and antioxidant and further more isolation in future.

Material and Methods

Plant materials and preparation of extract

The plant Bottle gourd (*Lagenaria siceraria*) and Neem (*Azadirachta indica*) were obtained from the St. Columba's College garden and used for the analysis.

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Neem (*Azadirachta indica*) and Bottle gourd (*Lagenaria siceraria*) were dried fruit, leaves and stem were separately ground to coarse powder using grinder. The powdered sample (approx.50g.) was then put in the Soxhlet apparatus and extracted utilizing different solvents (ethanol & chloroform). The extracts so obtained were further processed using Rotaory evaporator. The extracts were then collected, kept in petridish and stored in a dessicator at room temperature (AOAC, 2000).

Qualitative analysis of phytochemical

Screenings for detection of various phytochemicals were done by qualitative chemical tests of the crude plant extracts. The extracts obtained were subjected to the preliminary investigation for detection of carbohydrates, proteins-free amino acids, lipids, steroids, alkaloids, tannins, flavonoids, phenolic compounds, saponins, triterpenes and terpenoids, following the methods similar to *Khandelwal (1995) and Sinha and Dogra (1985)*.

Antimicrobial Activity

The antimicrobial susceptibility tests were carried out using standard methods described as disc diffusion method. The plates were prepared by pouring molten media into sterile

petri plates, and then plates were allowed to solidify. Now the inoculum suspension was spread uniformly and the inoculum was allowed to dry. The loaded disc of different concentration was placed on the surface of medium and the compound was allowed to diffuse, the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured as described by *Bauer et al., (1966)*.

DPPH free radical scavenging assay

The antioxidant activity of extracts on the basis of scavenging ability of the stable radical DPPH was determined. The ascorbic acid was used as reference standard. Varying concentrations of the extract was taken in the test tubes and distilled water was added to make it to 0.1m mol. The 2ml of DPPH solution (0.1mM in methanol) was added in each test tube, mixed well and incubated in dark at room temperature for 30mins. Absorbance of the mixture was measured at 517nm using spectrophotometer as described by *Williams et al., (1995)*.

Results and Discussion

Table 1: Qualitative analysis of Ethanolic Extract of Bottle gourd (*Lagenaria Siceraria*) & Neem (*Azadirachta Indica*) (+ positive result, - negative result)

| S. No | Test | Fruit(<i>Lagenaria siceraria</i>) | Leaves (<i>Lagenaria siceraria</i>) | Leaves(<i>Azadirachta indica</i>) | Stem(<i>Azadirachta indica</i>) |
|-------|----------------|-------------------------------------|---------------------------------------|-------------------------------------|-----------------------------------|
| 1 | Carbohydrate | + | + | + | + |
| 2 | Polysaccharide | - | - | - | - |
| 3 | Protein | + | + | + | + |
| 4 | Steroid | - | + | + | - |
| 5 | Flavonoid | + | - | + | + |
| 6 | Tannins | + | + | + | - |
| 7 | Terpenoids | + | - | + | - |
| 8 | Triterpenes | + | - | + | + |
| 9 | Lipid | + | - | + | + |
| 10 | Saponins | - | - | + | - |
| 11 | Amino Acid | + | - | + | - |

Table 2: qualitative analysis of chloroform extract of bottle gourd (*Lagenaria siceraria*) & neem (*Azadirachta indica*) (+ positive result, - negative result)

| S. No | Test | Fruit (<i>Lagenaria siceraria</i>) | Leaves (<i>Lagenaria siceraria</i>) | Leaves (<i>Azadirachta indica</i>) | Stem (<i>Azadirachta indica</i>) |
|-------|----------------|--------------------------------------|---------------------------------------|--------------------------------------|------------------------------------|
| 1 | Carbohydrate | + | + | + | + |
| 2 | Polysaccharide | - | - | - | - |
| 3 | Protein | + | + | - | + |
| 4 | Steroid | + | + | + | - |
| 5 | Flavonoid | + | + | + | + |
| 6 | Tannins | - | - | + | - |
| 7 | Terpenoids | - | + | + | + |
| 8 | Triterpenes | - | + | - | + |
| 9 | Lipid | - | - | + | + |
| 10 | Saponins | + | - | - | - |
| 11 | Amino Acid | - | + | + | - |

Phytochemicals such as steroids flavonoids, tannins, terpenoids, triterpenes, lipids, saponins, amino acid, etc., were present in the extract which may be responsible for the antioxidant as well as antimicrobial activity. But these secondary metabolites varied for each extract depending on

their parts used as well as solvents prepared, similar to *Tiwari et al., (2011)* who reported phytochemical analysis for alkaloids, glycoside, saponin, steroid, phenol, flavonoid, tannin, protein, and amino acids

Antibacterial activity

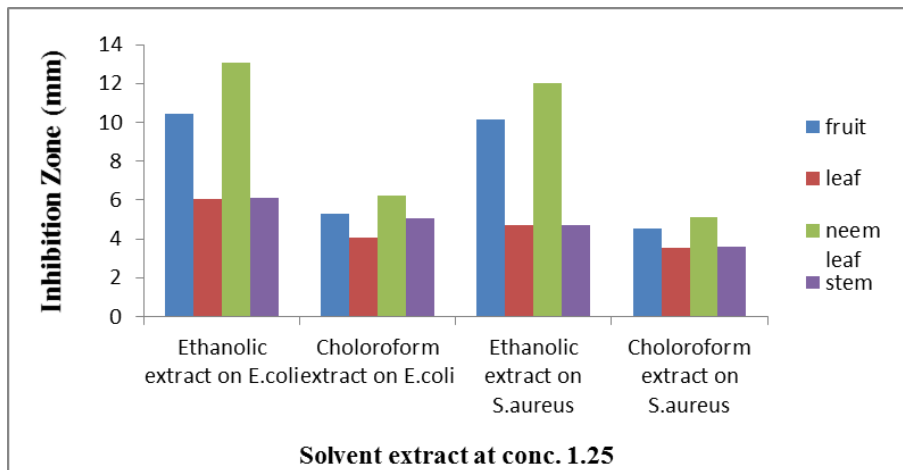


Fig 1: Zone of inhibition for ethanolic and chloroform extract of Bottle gourd (*Lagenaria siceraria*) and Neem (*Azadirachta indica*) on *E. coli* and *S. aureus*.

The ethanolic and chloroform extract showed significant antimicrobial activity and effectively inhibited the growth of *E. coli* and *S. aureus*. The ethanolic extract of Bottle gourd inhibited maximum zone of inhibition of 13mm for *E. coli* as compared to *S. aureus* which showed almost similar of 12mm zone of inhibition. This difference in the ethanolic extract displayed that the defensive mechanism of Bottle gourd is highly effective against a broad spectrum of gram positive and gram negative bacteria, hence as Neem being antibiotic from past the Bottle gourd also can serve the purpose. While the chloroform extract for both the plants displayed quiet similar effects towards antimicrobial properties. Similar to the antimicrobial properties of cucurbit taken by, Umadevi *et al.*, (2011)

also stated cucurbita has been very effective as anti- depressant and antioxidant. Which showed that *Lagenaria siceraria* renders antibacterial, antifungal, antioxidant and anti- inflammatory effects. Another study done by Joshi *et al.*, (2011) was also successful in identifying candidate plant with different antimicrobial activity, the investigation showed potent antimicrobial properties of *Ocimum sanctum*, it was found that different parts of the plant that is stem, fruit, flower and leaves have different antimicrobial properties which also showed significant differences with the change in solvent systems.

Free radical scavenging activity

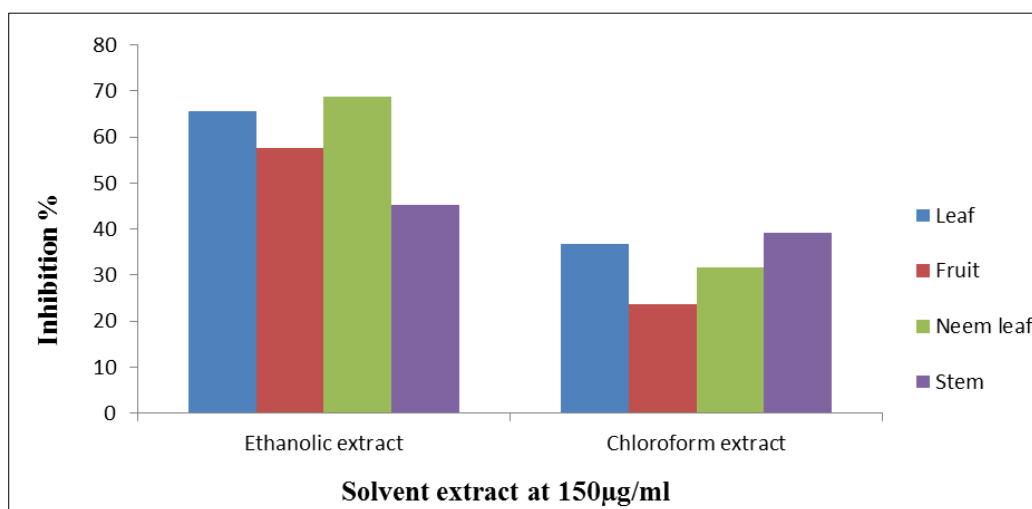


Fig. 2: Free radical scavenging effect of ethanolic extract of Bottle gourd (*Lagenaria siceraria*) and Neem (*Azadirachta indica*)

The free radical scavenging activity which was performed using DPPH assay displayed that ethanolic extract of *Lagenaria siceraria* at 50µg/ml concentration have 23.4% scavenging activity for fruit and at same concentration leaves had 19% scavenging activity while *Azadirachta indica* stem displayed 17.6% scavenging activity and leaves had 23.8% scavenging activity. Scavenging activity of chloroform extract displayed that *Lagenaria siceraria* at 50µg/ml concentration have 14.7% scavenging activity for fruit and at same concentration leaves had 10.9% scavenging activity while *Azadirachta indica* leaves displayed 13.1% scavenging activity and stem had 16.7% scavenging activity. Similar studies done by Rachh *et al.*, (2009) reported that according to the pharmacological antioxidant activity the acetone extract of fruit epicrap of *Lagenaria siceraria* fruit have maximum antioxidant activity against *in vitro* models using DPPH. The fresh juice of the fruit shows the antiradical activity. Rahman *et al.*, (2012) also reported using 2, 2-

diphenyl-1-picrylhydrazyl (DPPH) scavenging methods to determine the antioxidant activity of raw garlic extract that showed a color change from deep violet to yellow, indicating antioxidant activity and reported that the raw garlic can be a source of antioxidant based on the results of the DPPH scavenging analysis.

Conclusion

It implies that ethanolic and chloroform extract of the samples possess favourable results as well as non- favourable for both Bottle gourd (*Lagenaria siceraria*) and Neem (*Azadirachta indica*) depending upon the test conducted and their properties. The Neem (*Azadirachta indica*) plant which has served in fulfilling various pharmacological purposes from ancient was when compared to Bottle gourd (*Lagenaria siceraria*) reported that Bottle gourd (*Lagenaria siceraria*) can serve the purpose of antioxidant, antimicrobial properties, etc. as well. The variation occurred maybe

due to the solubility nature of the solvent used, variable type and quantity of phytochemicals present in that solvent extract. Hence further more deep analysis can be carried out to bring new findings in purpose to serve the future.

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