PRELIMINARY PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL STUDIES OF FLACOURTIA JANGOMAS FRUITS

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INTRODUCTION
India has an ancient heritage of traditional medicine. Indigenous medicines are based on various systems including Ayurveda, Siddha and Unani. The evaluation of these is mainly based on phytochemical, pharmacological and allied approaches including various instrumental technique like chromatography, microscopy and others. Lot of efforts have been taken by the government and private sectors for the development of the traditional system based on these three methods [1].

Flacourtia jangomas is a plant belongs to the family of Flacouriaceae growing in various parts of the world. It has been used as a traditional medicine for the treatment of different diseases in India [2]. Apart from its medicinal use, the plant is used for its edible fruits and lumber [3].

Distribution
Flacourtia jangomas is a tree from the lowland areas cultivated in Southeast and East Asia [5]. The species is cultivated mainly in villages and then it was distributed throughout tropical regions in East Africa and tropical Asia. The species is also found in the warmer coastal districts of eastern Australia.

MATERIALS AND METHODS
Collection and extraction of plant material
The fruits of Flacourtia jangomas were collected from the localities in Guwahati, Kamrup (M), Assam during the month of March 2017. The F. jangomas were collected, shade dried, powdered mechanically and sieved through no. 20 mesh sieve. About 100g of powdered fruits is extracted successively with petroleum ether (PEL), 60-80°C and then with dichloromethane (DCM), n-butanol (NB), ethyl acetate (EEL) and methanol (MEL). The extract collected was filtered and evaporated using rotary evaporator and stored in vacuum desiccators. The percentage yield of the extract is listed in table 1.

Table 1: Percentage yield of various extracts

| Extracts | % yield |
|----------|---------|
| PEL      | 6.7     |
| DCM      | 8.4     |
| NB       | 9.8     |
| EEL      | 9.5     |
| MEL      | 9.2     |

PEL: petrol ether extract of F. jangomas fruits; CEL: chloroform extract of F. jangomas fruits; EEL: ethyl acetate extract of F. jangomas fruits; MEL: methanolic extract of F. jangomas fruits.

Phytochemical screening of the extracts
Phytochemical screening were carried out for NB, DCM, MEL extract of F. jangomas for the presence of phytochemical constituent like alkaloid, glycoside, tannin, amino acid, steroid, protein, flavonoid etc [6, 7].
In vitro antioxidant studies

Reducing power

1 ml of methanolic extract (100-400 μg/ml), standard ascorbic acid dilutions (20-100 μg/ml) and control sample (1 ml distilled water instead of sample solution) was mixed with 2.5 ml phosphate buffer solution (pH 6.6) and 2.5 ml potassium ferricyanide (1%). The final mixture was properly mixed and incubated at 50 °C for 20 min. After incubation, the reaction mixture was rapidly cooled and mixed with 2.5 ml of 10% trichloroacetic acid. It was centrifuged at 3000 rpm for 10 min. About 2.5 ml of the supernatant was taken, and 2.5 ml distilled water and 0.5 ml of ferric chloride (0.1%) were added; it was mixed well and allowed to stand for 10 min. The absorbance was measured at 700 nm [8].

Thiobarbituric acid method

The test was conducted according to the method of Kikuzaki and Nakatani (1993) [9]. The same samples prepared for FTC method were used. To 2.0 ml of the sample solution, 1.0 ml of 20% aqueous trichloroacetic acid (TCA) and 2.0 ml of aqueous thiobarbituric acid (TBA) solution were added. The final sample concentration was 0.02% w/v. The mixture was placed in a boiling water bath for 10 min. After cooling, it was then centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured at 532 nm. Antioxidant activity was recorded based on the absorbance of the final day of the FTC assay. Both methods (FTC and TBA) described antioxidant activity by percent inhibition:

\[
\% \text{ Inhibition} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100
\]

Antimicrobial activity

Nutrient agar media

Bacteriological media is a wide range of types. Nutrient Agar is a complex medium as it contains ingredients with unknown amounts or types of nutrients. Nutrient Agar contains Beef Extract (0.3%), Peptone (0.5%), Agar (1.5%) and sodium chloride (0.5%) in water. Beef Extract is a commercially prepared dehydrated form of autolyzed beef and is supplied in the form of a paste. Peptone is the casein (milk protein) that has been digested with the enzyme pepsin. Peptone is dehydrated and supplied as a powder. Peptone and Beef Extract contain a mixture Amino and peptides of acids. Beef Extract also contains water soluble digest products of all other macromolecules (nucleic acids, Fats, polysaccharides) as well as vitamins and trace minerals. Although we know and can define Beef Extract in these terms, each batch cannot be chemically defined. There are many media ingredients which are complex yeast extract, tryptone, and others. The advantage of complex media is that they support the growth of wide range of microbes. Agar is purified from red algae in which it is an accessory polysaccharide (polysaccharolistic acid) of their cell walls. Agar is added to microbial media only as a solidification agent. Agar for the most purpose is not nutrient value. Agar is an excellent solidification agent because it dissolves at near boiling but solidifies at 45 °C. Thus, one can prepare molten (liquid) agar at 45 °C, mix cells with it, and then allow it to solidify thereby trapping living cells. Below 45 °C is a solid and remains so as the temperature is raised melting only when >95 °C is obtained.

Procedure

- Beef Extract, peptone were accurately weighted out and in 1000 ml of distilled water beef extract, peptone are added.
- PH was checked by using pH meter (pH 7.4), and finally, Agar was added to the flask.

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\begin{array}{|c|c|c|c|}
\hline
\text{Name of the test} & \text{Methanolic extract} & \text{N-butanol extract} & \text{DCM extract} \\
\hline
\text{Glycoside} & + & + & - \\
\text{Alkaloid} & + & - & + \\
\text{Flavonoid} & + & + & + \\
\text{Protein} & - & + & + \\
\text{Amino acid} & - & - & + \\
\text{Tannin} & + & + & + \\
\text{Steroid} & + & + & + \\
\text{Phenolics} & + & + & + \\
\hline
\end{array}
\]

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Methanolic, n-butanol and DCM extract Flacourtia jangomas fruits showed the presence of phenolics, steroids, and flavonoids also glycosides (except DCM) shown in table 1. Medicinal plants are a great source of active constituents for the development of new therapeutic compounds.
**In vitro antioxidant activity**

Table 3: Reducing power assay

| S. No. | Sample                     | Activity | 20 μg/ml | 40 μg/ml | 60 μg/ml | 80 μg/ml | 100 μg/ml |
|--------|----------------------------|----------|----------|----------|----------|----------|-----------|
| 1      | Standard: (Ascorbic acid)  | Standard | 1.636±0.022 | 2.389±0.020 | 3.127±0.030 | 3.459±0.017 | 4.124±0.040 |
| 2      | Test (DCM extract)         | RP       | 1.435±0.020 | 2.010±0.018 | 3.022±0.022 | 3.154±0.012 | 4.088±0.039 |
| 3      | Test (NB extract)          | RP       | 1.468±0.019 | 2.330±0.020 | 3.124±0.025 | 3.359±0.010 | 4.115±0.030 |
| 4      | Test (EA extract)          | RP       | 1.226±0.011 | 1.505±0.014 | 3.026±0.012 | 3.227±0.006 | 4.005±0.022 |
| 5      | Test (Methanol extract)    | RP       | 1.433±0.020 | 2.220±0.022 | 3.044±0.026 | 3.339±0.015 | 4.006±0.042 |

IC50 value in reducing power assay 254.5 μg/ml for extract and 43.7 μg/ml for standard (table 5). Substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form a ferrous ferrocyanide complex that has an absorption maximum at 700 nm. These assays are known as a robust and useful method for measuring a wide concentration range of antioxidant activities and capacities [10].

**Thiobarbituric assay**

The test was conducted according to the method of Kikuzaki and Nakatani (1993) [9]. The same samples prepared for FTC method were used. To 2.0 ml of the sample solution, 1.0 ml of 20% aqueous trichloroacetic acid (TCA) and 2.0 ml of aqueous thiobarbituric acid (TBA) solution were added. The final sample concentration was 0.02% w/v. The mixture was placed in a boiling water bath for 10 min. After cooling, it was then centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured at 532 nm. Antioxidant activity was recorded based on the absorbance of the final day of the FTC assay.
Antimicrobial activity

The Methanolic extract, ethyl acetate extract and N-butanol extract of *Flacourtia jangomas* were tested for antibacterial activities against a number of both gram positive and gram negative bacteria. Standard Chloramphenicol discs (10 µg/disc) were used for the comparison purpose. The antimicrobial activities of the methanolic extract, ethyl acetate extract and N-butanol extract from *Flacourtia jangomas* were tested in the present study. The results were given gradually in table 4, table 5 and table 6.

### Table 4:

| S. No. | Sample                        | Activity | 20 µg/ml | 40 µg/ml | 60 µg/ml | 80 µg/ml | 100 µg/ml |
|-------|-------------------------------|----------|----------|----------|----------|----------|-----------|
| 1     | Standard: (Ascorbic acid)     | TBA      | 32.35±0.001 | 49.67±0.002 | 55.22±0.001 | 65.60±0.05 | 75.47±0.01 |
| 2     | Test (DCM extract)            | TBA      | 31.23±0.011 | 44.34±0.002 | 53.45±0.101 | 63.45±0.04 | 72.34±0.011 |
| 3     | Test (NB extract)             | TBA      | 32.44±0.101 | 45.10±0.001 | 54.12±0.005 | 64.34±0.05 | 74.33±0.02 |
| 4     | Test (EA extract)             | TBA      | 30.46±0.002 | 41.20±0.004 | 50.32±0.001 | 61.23±0.07 | 71.23±0.031 |
| 5     | Test (Methanol extract)       | TBA      | 29.23±0.012 | 40.08±0.121 | 49.34±0.022 | 60.11±0.05 | 70.12±0.22 |

### Table 5: MIC of the methanolic extract

| Test sample     | Concentration (µg/ml) | Diameter zone of inhibition (mm) |
|-----------------|-----------------------|----------------------------------|
| Methanolic Extract | 10 | 1.1 | 2.2 |
|                 | 20 | 1.5 | 3.1 |
|                 | 30 | 2.3 | 5.5 |
|                 | 40 | 4.2 | 7.1 |
|                 | 50 | 6.1 | 7.9 |
|                 | 60 | 6.9 | 9.3 |
|                 | 70 | 7.5 | 9.7 |
|                 | 80 | 8.7 | 10  |
| Standard        | 10 | 15.1| 15.1|

![Fig. 3: Effect on antimicrobial activity of methanolic extract of F. jangomas](image)  

### Table 6: MIC of the ethyl acetate extract

| Test sample     | Concentration (µg/ml) | Diameter zone of inhibition (mm) |
|-----------------|-----------------------|----------------------------------|
| Ethyl Acetate   | 10 | 0.6 | 2.0 |
|                 | 20 | 1.0 | 2.6 |
|                 | 30 | 1.9 | 3.9 |
|                 | 40 | 2.1 | 6.0 |
|                 | 50 | 4.9 | 6.7 |
|                 | 60 | 5.4 | 8.8 |
|                 | 70 | 6.3 | 9.0 |
|                 | 80 | 7.9 | 9.7 |
| Standard        | 10 | 16.1| 16.1|
Table 7: MIC of the n-butanol

| Test sample          | Concentration (µg/ml) | Diameter zone of inhibition (mm) |
|----------------------|-----------------------|----------------------------------|
|                      |                       | Gram-ve bacteria (E. Coli)   | Gram+ve bacteria (S. aureus) |
| N. butanol extract   | 10                    | None                            | 1.2                           |
|                      | 20                    | 1.2                              | 2.4                           |
|                      | 30                    | 1.5                              | 2.9                           |
|                      | 40                    | 1.8                              | 3.7                           |
|                      | 50                    | 3.1                              | 4.6                           |
|                      | 60                    | 3.8                              | 6.4                           |
|                      | 70                    | 4.5                              | 7.3                           |
|                      | 80                    | 6.8                              | 8.1                           |
| Standard             | 10                    | 14                               | 14                            |

DISCUSSION

Various chemical constituents such as glycosides, flavonoids, phenolics and steroids were found. Phenolics, steroids and flavonoids were present in all the extracts whereas proteins, alkaloids and amino acids are absent. Free radicals are harmful as they can participate in superfluous side reactions due to their chain reaction properties which resulting in cell damage, blemishing of food, degradation of various materials like rubber, gasoline etc.

Antioxidants are molecules which can halt these chain reactions by removing free radical intermediates. Phenolic compounds are responsible for antioxidant properties due to their ability to donate electron resulting in the conversion of highly reactive free radicals to nonreactive stable molecules.

Phenolic compounds may be of three types-non-flavonoids like hydroxybenzoic acid, flavonoids like flavones, flavonols, flavanones etc and type three is tannins. The n-butanol extract of Flacourtia
jangomas showed a high amount of antioxidant activity in reducing power assay and thiobarbituric assay which is because of the presence of flavonoids. N-butanol extract exhibited a proportional increase of absorbance along with the concentration which proves the presence of some active compounds those are capable of reacting with free radicals and converting them to stable non-reactive form as well as terminating chain reactions. The indiscriminate use of antibiotics has developed many resistant micro-organisms creating immense clinical problems in the treatment of infectious diseases such as those caused by multi-drug resistant S. aureus and E. coli. Therefore there is a need to develop alternative antimicrobial agents for the treatment of these infectious diseases. There are many investigations carried on the aerial parts (leaves) of Flacourtia jangomas by various workers, however, not many studies are available on the antimicrobial and pharmacological potential of Flacourtia jangomas fruits.

CONCLUSION

From these investigations, it may be concluded that the n-butanol fruits extract of F. jangomas showed potential antioxidant, in different established models. These results also justify the use of leaves in traditional medicines. It is important to isolate and characterize the active compounds responsible for these activities through LC-MS and other advanced techniques.

CONFLICT OF INTERESTS

Declare none

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