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Phyto-Chemical Screening and Anti Listerial Activity of *Annona Muricata* (L) Leaf Extract

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Abstract

Annona muricata is a potent medicinal plant with wide range of therapeutic activity. Various parts of this plant have immense beneficial and curative properties. The phyto-chemical screening of the leaf extract was performed using aqueous and methanolic extracts that showed the presence of all major primary and secondary metabolites and anti-oxidant trait. The aqueous extract was then subjected to anti listerial activity that showed significant results.

Keywords: *Annona muricata*; Anti-oxidant; Secondary metabolites

Introduction

Bio-prospecting is the systematic scientific tool for the search of genes, natural bio-active compound that has the potential for the product development by biological observation and bio-physical, bio-chemical and genetic methods, without disruption to nature. Most of the bio-prospecting is performed on a small scale by numerous academic and research groups throughout the world. It has helped to explore various plants of immense use to mankind.

Natural remedies from medicinal plants are found to be safe and effective. Many plant species have been used locally as medicine to treat various ailments. Bio-active compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries. According to the WHO survey, 80% population depends upon the traditional medicines for primary health care needs. It suggested in improving the technologies for cultivation of medicinal plants. It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to traditional herbal medicine [1-5]. *Annona muricata L.* (Soursop) is a naturally occurring plant, traditionally used to treat various ailments including cancer. It belongs to the family *Annonaceae* and is widely distributed in India and Central America. Fruits of *Annona muricata*, also known as Graviola in South America are taken internally for worms, parasites, fever, increase mother's milk production after child birth and as an astringent for diarrhoea and dysentery. The plant is also reported to have good antioxidant property. Furthermore, the leaves of the plant are found to be anti-spasmodic, hypotensive and are rich in annonaceous acetogenins. These leaves are traditionally used to prevent and treat arthritis, asthma, bronchitis biliary disorder, diabetic, heart diseases, hypertension, worm disease, liver disorder, malaria, rheumatism, sedative, tumor, and cancer. The leaves are also used for the treatment of several types of diseases caused by bacteria such as pneumonia, diarrhea, urinary tract infection, and other kinds of skin diseases. This plant has numerous benefits for human life due to high nutrient value. In the food industry soursop can be processed into jam, fruit juice, syrup. Soursop leaves contain flavonoid, tannin, alcaloid, saponin, calcium, phosphor, carbohydrate, vitamin A, B and C, phytosterol, calcium oxalate etc. Plant and plant-derived compounds are alternative sources for treating microbial infections. Various other plants from this family have also been reported for their cytotoxic potential [6-13]. Keeping in view its promising therapeutic potential, the leaf was used for phyto-chemical screening, anti-oxidant properties and using the extract to explore its anti listerial properties.

Materials and Methods

Sample collection and extraction

Plant sample (*Annona muricata*) was collected from Agricultural College and Research Institute Agricultural University, Madurai, Tamil Nadu, India. The plant was washed thoroughly in tap water. The leaf of the plant were air dried in shady place and powdered.

25 g of the powder was packed in soxhlet assembly and extracted for 16 hours by 500 ml methanol. 25 g of dried powder was macerated with 100 ml of pure distilled water in a conical flask and shaken at room temperature for 24 hours and filtered through Whatman No.1 filter paper. The crude extracts were taken and subjected to qualitative photochemical screening and anti listerial (Figure 1).

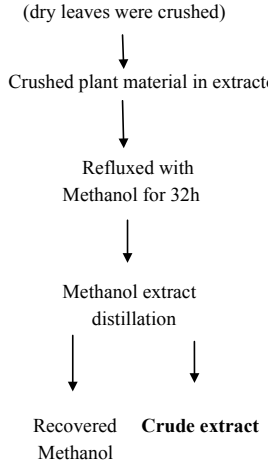

Figure 1: A chart depicting the process to get the extract of the solvents from the plant material

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Extraction

The phyto-chemical screening tests of the extracts for qualitative analysis of primary and secondary metabolites was carried out as per the standard procedure [14-16]. The quantitative analysis of Vitamin C and Vitamin E was done by standard reference method [17].

Phyto-chemical screening

The extract obtained was tested for alkaloids, saponins, sterol and terpenes, tannins, proteins and glycoside as per the methods reported earlier [15-17].

Test for alkaloids: A portion of the extract was made acidic with dilute sulphuric acid and the acidic extract was divided into two parts. With Mayer's reagent it gives while ppt for positive test. With Dragendorff's reagent it gives orange brown ppt. for the positive test.

Test for saponins: A small amount of the extract was boiled with water and allowed to cool. It was shaken vigorously in a test tube and left for a few minutes. The formation of persistent honey comb like froth was taken as a positive test.

Test for sterols and terpenes: A small amount of the extract was evaporated to dryness and extract was dissolved in 3 ml of chloroform. The filtrate was treated with three drops of a mixture of concentrated sulphuric acid and acetic anhydride. The production of different shade of color was recorded as a positive test.

It was furthered verified by Libermann Buchard test. A small portion of the extract was treated with hot acetic anhydride, cooled and then few drops of concentrated sulfuric acid were added, Production of bluish green color confirmed sterol while violet or pink for terpene.

Test for tannins: A small amount of the extract was treated with 5% ferric chloride solution and the production of green to blue color was taken as a positive test for tannins.

Test for proteins: Addition of very dilute copper sulphate to alkaline solution of protein gives red to violet solution that confirms protein by Biuret test. Protein produces yellow orange color when warmed with concentrated nitric acid and color gets orange when made alkaline in Xantho proteins test. Millon's reagent gives white ppt. when a solution of mercuric nitrate containing nitrous acids is added to a protein solution.

Test for carbohydrate: Molish test is positive when on treatment with alpha napthol and concentrated sulphuric acid the extract gives purple color. Reduction of Fehling's solution is seen when in a solution of carbohydrate equal quantity of Fehling A and B are added. After heating brick red ppt. is obtained. In Benedict's test, the test solution gave yellow or reddish brown precipitate with Benedict's reagent after boiling on water bath.

Anti listerial activity

The anti Listerial activity for the leaf extract was studied on *Listeria monocytogenes* MTCC 657, a representative model organism used to screen the antibacterial activity procured from Microbial Type Culture Collection, Chandigarh, Punjab, India. To study the effect of leaf extract, the organism was cultured in nutrient broth at 37°C and then sub-cultured after every 24 hrs so as to maintain it in log phase. For all the experiments, actively proliferating log phase cells were taken and the anti listerial activities of leaf extract were studied by growing the cells at the final concentrations of 0.25 mg/ml and 0.50 mg/ml in total of 2 ml culture media. $1x10^5$ cells of *Listeria monocytogenes* (as counted by haemo cytometer) were used per ml of the media as inoculum. Growth of cells was measured by Optical Density (OD) measurements at 600 nanometer. Experiments were conducted with *Listeria monocytogenes* grown at 37°C in presence of leaf extract complexes at the final concentrations of 0.25 mg/ml and 0.50 mg/ml. Cell turbidity was measured after 24 hours at 600 nanometers. The assay medium components are given in the Table 1[18-23]. The medium was prepared by dissolving the content in 1000 ml distilled water at pH 7.0. The distribution and sterilization of medium was done as per experimental requirements.

Results and Discussion

Phyto-chemical screening

The results obtained by qualitative phyto-chemical screening for primary and secondary metabolites in the leaf extracts have been summarized below in Table 2.

The result obtained above shows that leave has phyto-chemical constituents like carbohydrate, protein, fats, alkaloids, terpenoids, flavonoids, saponin, tannin and glycosides etc. The results obtained by quantitative analysis of the aqueous extract for Vitamin C and E is as follows:

The presence of non-enzymatic anti-oxidant components like Vitamin C and E in the leaf is high content reveals that the leaves are effective with significant neo-plastic potential (Table 3).

Anti listerial activity

The biological activity for the leaf extract was studied on *Listeria monocytogenes* MTCC 657, a representative model organism used to screen the anti-listerial activity. Growth of cells was measured by Optical Density (OD) measurements at 600 nanometer. Percentage inhibitions of leaf extract are presented in Table 4. Highest Inhibitory activity (up to 78.8%) has been found which shows very promising results as an anti listerial agent. Zone of inhibitions of leaf extract in mm is 14 mm by .25 mg/ml and 22 mm .050mg/ml which is again a good inhibition against *Listeria* (Tables 4 and 5 and Figures 2 and 3) [18-23].

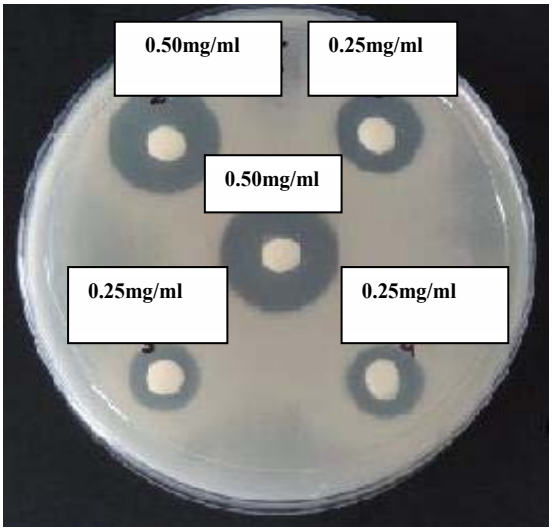

Figure 2: The anti listerial activity of leaf extract

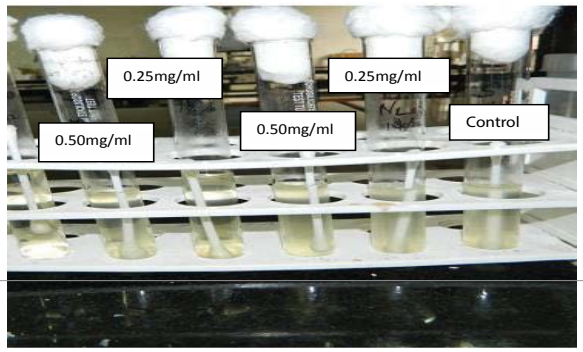

Figure 3: Percentage inhibition after adding leaf extract.

Ingredients	Quantity (g/l)
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15
pH	7.0 ± 0.2

Table 1: Composition of the nutrient medium

Sr	Plant Constituents	Test Performed	Aqueous Extract	Methanol Extract
1	Carbohydrate	Benedict test Molisch test	+ +	+ +
2	Protein and Amino acids	Ninhydin test Biuret test	+ +	+ +
3	Fats and Fatty acids	Phenophthalin test Spot test	+ +	+ +
4	Alkaloid	Mayer test Wagner test Dragondroff test	- - -	+ + -
5	Terpenoid	Salkovaski test	+	-
6	Steroid	Liberman Buchard test	+	+
7	Flavonoid	Shinoda test Alkaline reagent test	+ -	+ -
8	Tannin and Phenolic compound	Ferric chloride test Lead acetate test	- +	- +
9	Saponin	Froth test Lead acetate test	+ +	+ +
10	Glycoside			
10a	Cardiac glycoside	Keller killani test	+	+
10b	Anthraquinone glycoside	Ammonia test	+	-
10c	Coumarin glycoside	Alkaline test	+	-

Table 2: Phyto-chemical screening of the *Annona muricata* leaf extract

S. No	Anti oxidant components	Aqueous Extract (mg%)
1	Vitamin C	65.0
2	Vitamin E	07.0

Table 3. Anti oxidant property of aqueous extract.

Sr. No.	Time	Average Percentage of inhibition	
		Concentration (0.25 mg/ml)	Concentration (0.50 mg/ml)
1	24h	43.9	50.0
2	36h	52.0	63.0
3	48h	70.0	78.8

Table 4. Anti Listerial activity of aqueous leaf extract

S.No.	Concentration of aqueous leaf extract (mg/ml)	Zone of Inhibition in (mm)
1	0.25	14.0
2	0.50	22.0

Table 5. Inhibition zones at various concentrations.

Conclusions

The aqueous leaf extract and the methanolic extract when subjected to phyto-chemical screening showed the presence of most of the major primary and secondary metabolites. The leaf extract showed significant anti-oxidative properties. The Anti-listerial activity of leaf extract revealed the anti-listerial potential of the leaf. The results obtained from zone of inhibition method are in same lines with those observed from turbidity method. Zone of inhibition (Table 5) increases with increasing concentration of extract. The result obtained by anti listerial assay clearly suggests that the aqueous extract is effective against *Listeria monocytogenes* bacteriocins as they successfully inhibited *L. monocytogenes* that caused spectic abortion, newborn and adult septicemia, listeriosis, meningitis and meningo-encephalitis in immune-deficient persons. It shows that the extract is effective against *L. monocytogenes* a food pathogen.

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