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Methyl Ester of hexadecanoic acid from Annona muricata leaf extract and its Antibacterial Activity

NATIONAL COLLEGE TRICHINOPOLY

Introduction

- Herbal remedies from plants having medicinal properties have been used traditionally in many parts of the world (Adewole and Caxton-Martins, 2006).
- World Health Organization (WHO) estimated 80% of the developing world's Population depend on traditional medicine and recognized more than 20,000 species of medicinal plants.
- Modern medicines has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening (Boopathi and Sivakumar, 2011).

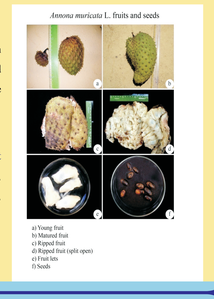
- Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine, food supplements, pharmaceutical and chemical entities for synthetic drugs (Ncube et al.,2008).
- Screening of active compounds has led to the invention of new herbal drugs for protection and treatment of various diseases (Turger and Usta, 2008).
- The present research work "*In vitro* Propagation, Phytochemical, Antimicrobial, and Larvicidal activities of *Annona muricata* L." with the following objectives:

1. To isolate and identify the phytochemical compounds by using preliminary, TLC, UV-Visible, FTIR and GC-MS methods.
2. To screen the antibacterial, antifungal and antioxidant activities.
3. To determine the Minimum inhibitory Concentration (MIC) for bacterial and fungal pathogens

DESCRIPTION OF PLANT

- *Annona muricata* L. plant is commonly known as soursop or graviola, belongs to the family Annonaceae.
- *A. muricata* L. (Annonaceae), a medicinal plant has been traditionally used to treat headaches, hypertension, cough, asthma, antispasmodic, sedative and nervine for heart condition (Lans, 2006).

- It is a typical tropical tree with heart shaped edible fruits and widely distributed in most of the tropical countries (De Feo,1992).
- The fruit pulp contain rich amount of micronutrients like retinol, ascorbic acid, anthocyanin, flavonoids and tannins.



- *A. muricata* has been placed under the category of rare / endangered plants.
- The threat could be attributed to anthropogenic factors like habitat destruction, availability of pollinators, seed setting and seed viability.
- Due to these factors, the plant requires a lot of attention for conservation.

2. PHYTOCHEMICAL STUDIES

Preliminary phytochemical studies (Brinda et al., 1981)

Detection of Bioactive compound (Bothast and Hesseltine, 1975)

- TLC analysis of crude leaf extract for isolation of bioactive compounds.
- UV and FTIR analysis for identification of bioactive compounds
- GC-MS analysis for the identification of compounds.

3. ANTIMICROBIAL STUDIES

- *In vitro* antibacterial activity of aqueous leaf extract was conducted by disc diffusion method with increasing concentration of 50, 100 , 200 and 400 µg/mL in disc. Positive control – Streptomycin (20 µg/disc).
- *In vitro* antifungal activity of the aqueous leaf extract was carried out by pour plate method with 5, 10, 15 mg/ml. Positive control – Bavistin (0.5 mg/mL).

4. ANTIOXIDANT ASSAY

- Measurement of a,a-Diphenyl-ß-picrylhydrazyl (DPPH) radical scavenging activity (Oktay et al., 2003).

$$\% \text{ scavenging effect} = \frac{A_o - A_1}{A_o} \times 100$$

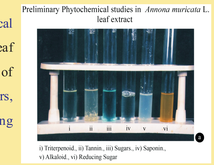
A_o – Absorbance of control

A_1 - Absorbance of Sample

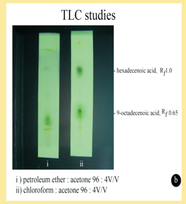
RESULTS

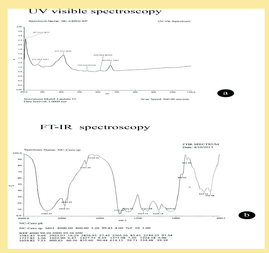
2. PHYTOCHEMICAL STUDIES

- The preliminary phytochemical analysis of *A. muricata* leaf extract revealed the presence of Triterpenoids, Tannins, sugars, saponins, alkaloid and reducing sugars.

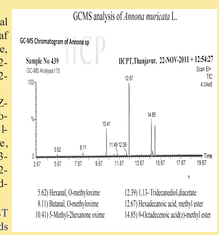


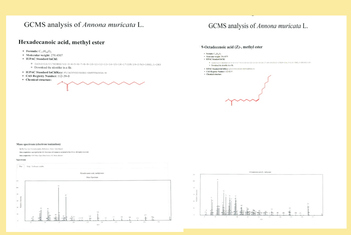
- Further TLC, UV-Vis spectroscopy, FTIR, and GC-MS analyses revealed the presence of major bioactive compounds such as Methyl ester of hexadecenoic acid and Methyl ester of 9-octa decenoic acid.

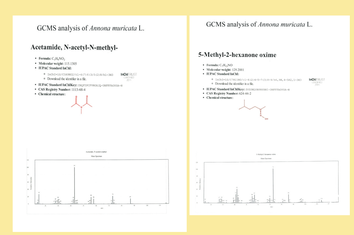




- The other phytochemical compounds of *A. muricata* leaf extract: Hexanol-O-methyloxime, Butanal-O-methyloxime, 2-propanone oxime, 5-methyl-2-hexanoneoxime,1,2 Ethanediamine, 2(Z-aminoethyl),butanamide 4-cyano-N-Methyl, 1,13-Tridecanodiol-diacetate, 10-undecen-1-yl acetate, Acetamide, N-acetyl-N-methyl, 3-cyclohepten-1-one, 5-Hexen-2-one-O-methyloxime, Acetic acid-2-methylpropylester.
- The detection employed the NIST (National Institute of Standards and Technology) library.



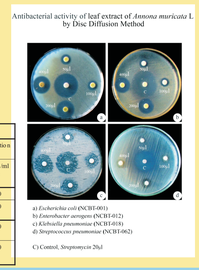




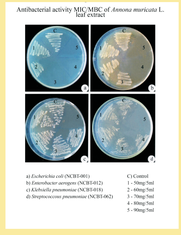
3. ANTIMICROBIAL STUDIES

- Antibacterial activities of *A. muricata* leaf extract is effective against pathogenic bacterial strains such as *Escherichia* coli, *Enterobacter aerogens, Klebsiella pneumoniae* and *Streptococcus pneumoniae* at 200 and 400 µg/mL concentrations.

Bacterial strains	Zone of inhibition in mm		
	Control (20 µg/ml) in disc	Leaf extract Concentration (1mg/ml)	
		200 µg/ml in disc	400 µg/ml in disc
Escherichia coli	28.0	20.0	20.0
Enterobacter aerogens	30.0	18.0	18.0
Klebsiella pneumoniae	20.0	16.0	20.0
Streptococcus pneumoniae	22.0	10.0	10.0



- The lowest MIC values for bacteria 60 mg/5mL was recorded for *Escherichia* coli, for *Enterobacter aerogens* 70 mg/5mL.
- The highest MIC value was recorded for *Klebsiella pneumoniae* and *Streptococcus pneumoniae* 80 mg/5mL.



4. ANTIOXIDANT ACTIVITY

Measurement of a,a-Diphenyl–ß-picrylhydrazyl (DPPH) radical scavenging activity

Sample	Scavenging Effect %
BHT (Antioxidant)	8.5
Vitamin E (Antioxidant)	4.0
Annona muricata L. leaf extract	9.5

In conclusion

- *A. muricata* leaf extract with its potential bioactive compounds methyl ester of hexadecenoic acid and methyl ester of 9-octa decenoic acid will serve as a safe natural plant based antimicrobial agent, antioxidant property agent.

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THANK YOU