RAMCO INSTITUTE OF TECHNOLOGY

Rajapalayam – 626 117, Virudhunagar District, Tamil Nadu.

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International Conference on Smart Technologies and Applications

11th & 12th March-2022.

Proceedings

ICSTA-2022

ISBN No.: 978-93-5593-352-2

(ISBN) 978-93-5593-352-2

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Physics (ICSTA-2022)

Chemistry (ICSTA-2022)

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(ISBN)
978-93-5593-352-2

CHEMISTRY-080

EVALUATION OF CHEMICAL PROFILE, IN VITRO ANTIBACTERIAL AND ANTIOXIDANT ACTIVITES OF ESSENTIAL OIL FROM A. HETEROPHYLLUS (L) LEAVES

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Abstract: The present study examines the nature of phytoconstituents and *in vitro* antioxidant activity of essential oil of *A.heterophyllus* (L) leaves. GC-MS analysis of the essential oil of A.heterophyllus (L) leaves revealed the presence of 12 compounds. The major constituents are Tritetracontane (53.5%), Tetracontane,3,5,24-trimethyl-(16.7%), 2-Hexyl-1-octanol(11.2%), 1-Hentetracontanol (10%), 1-Hexacosene (4.2%) and Octadecane-1-(ethenyloxy)-(2.3%). The in vitro antioxidant activity was tested using DPPH radical assay showed a concentration dependent antiradical activity by inhibiting DPPH radical with EC_{50} value of 60.17 µg/ml (BHT as the control with EC_{50} value of 18.8 µg/ml). Further the essential oil of *A.heterophyllus* leaves showed significant dose dependent antibacterial activity compared with standard drug Ampicilin.

Keywords: GC/MS, A.heterophyllus, DPPH and antibacterial activity.

Introduction

Artocarpus heterophyllus is a species of tree of the mulberry family (Moraceae) is known by other names jackfruit (Eng.), Kathal, Panas (Hindi), Kanthal (Beng.), Palaa (Tamil), Phanas (Guj & Mar) & Chakka (Malayalam). It is native to Western Ghats of India, Malaysia and also found in central and eastern Africa, south-eastern Asia, the Caribbean, Florida, Brazil, Australia, Puerto Rico and many Pacific Islands [1]. Parts of the jackfruit plant such as stems, roots, leaves and fruit have medicinal properties. Specifically, jackfruit leaves also contain sapogenins, cycloartenone, cycloartenol, β-sitosterol, and tannin [2]. The leaves are useful in fever, boils, wounds and skin diseases. The young fruits are acrid, astringent, and carminative. The ripe fruits are sweet, cooling, laxative, aphrodisiac and also used as a brain tonic. The seeds are, diuretic, and constipating. The wood is nervine, antidiabetic, sedative and is useful in convulsions [3]. The extract of *A. heterophyllus* showed a broad spectrum of antibacterial activity [4]. The methanol, ethanol, acetone and aqueous extracts of ripe pulp shown to possess free radical scavenging effects in DPPH, FRAP and DMPD assays [5].

Materials and methods

A.Plant material

The leaves of *Artocarpus heterophyllus* was collected during the season June-July from Pollachi, Coimbatore district, Tamil Nadu, India. The plant material was identified by Department of Botanty, NGM College, Pollachi, The voucher specimen was stored in Chemistry department.

B. Isolation of the essential oil

The material was chopped at room temperature before hydrodistillation. Essential oil was extracted from the leaf by hydro distillation in a Clevenger's type apparatus. The leaf (500g) was boiled with water (500ml) for 3h in a 2L round bottom flask fitted with condenser. The oil released

(ISBN)
978-93-5593-352-2

from the residues was collected from the condenser, dried over anhydrous sodium sulphate and the amount measured. It was then stored at 4^0 C for GCMS analysis.

C. Analysis of the essential oils

GC/MS analysis was evaluated using a Perkin Elmer Clarus 680 GC coupled with Perkin Clarus 600 (EI) instrument. The compounds were separated in a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethyl polysiloxane, 30 m × 0.25 mm ID × 250µm df). Helium was used as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 280°C during the chromatographic run. The 1µL of extract sample injected into the instrument, the oven temperature was set as follows: 60 °C (2 min); followed by 210 °C at the rate of 3 °C min^{-1} and finally 280°C held isothermally for 15 min. The mass detector conditions were as follows: transfer line temperature 230 °C; ion source temperature 230 °C; ionization mode electron impact at 70 eV over a range of 50–650 amu , a scan time 0.2 sec; scan interval of 0.1 sec and the fragments from 40 to 600 Da.

D. Identification of phytoconstituents of EAEO

The components were identified by comparison of their mass spectra with those of the MS search (NIST& Wiley) as well as by comparison of their relative retention times either with those of reliable compounds or by comparing their relative retention index (RI) to sequence of n-alkanes or with literature standards (Adams, 2007). Quantification was done by external standard method using standardization curves produced by running GC analysis of illustrative compounds.

E. Anti bacterial activity

1. Zone of Inhibition method

The bacterial strains (*Streptococcus* sp., *B.cereus, A. hydrophila, V. cholerae* were inoculated in the nutrient broth under aspectic condition and incubated at 37 °C for 18 hours. After the incubation period, the test bacterial was swabbed on the nutrient agar plate using sterile cotton swab. In each of these plates, wells (10 mm) were cutout using sterile cork borer. The essential oil was dissolved in the solvent. Controls were maintained by loading same quantity of Ampicillin into the wells. Then the petri dishes were incubated at 37 °C for 14 hours. The anti bacterial activity was determined by measuring the zone of inhibition using vernier caliper in diameter. The zone of inhibition in diameter was observed and recorded in millimeter.

F. Free radical scavenging activity

1. DPPH radical scavenging activity

The free radical scavenging activity of the essential oil of *A. heterophyhllus* was measured with the stable radical 1, 1-diphenyl-2-picrylhydrozyl (DPPH) in terms of hydrogen donating or radical scavenging activity. A 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of essential oil of *A.heterophyhllus* solution was made for different concentrations (50,100, 250, 500 and 1000 µg/ml). After 30 minutes, the absorbance was measured at 517nm. Lower absorbance of the reaction showed higher activity. The control experiment was also carried with distilled water in place of the extract. Butylated hydroxy toluene (BHT) was used as a standard. The antioxidant activity of the extract was expressed as IC_{50}, which was defined as the concentration (in µg/ml) of extract that inhibits the formation of DPPH radicals by 50%.

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Radical Scavenging Activity was calculated using the following equation:
Scavenging effect % = $[(A_0-A_1)/A_0]$ x 100, where A_0 was the absorbance of the control and A_1 was the absorbance of the extract.

Results and Discussion

A. Chemical composition of essential oil

The volatile compound identified in the essential oil from the fresh leaves of A.heterophyllus *was shown in (Table 1). The chemical composition of essential oil was determined by using Gas Chromatography Mass Spectroscopy (GC-MS) technique. The components were identified based on the comparison of their relative retention time and mass spectra with those of Wiley 7N Library data (Adams, 2001) and their mass fragmentation. It resulted in the identification of twelve compounds from approximately 99.6% of the oil. The major constituents are Tritetracontane (53.5%), Tetracontane,3,5,24-trimethyl-(16.7%), 2-Hexyl-1-* octanol(11.2%), 1-Hentetracontanol (10%), *1-Hexacosene(4.2%) and Octadecane-1-(ethenyloxy)-(2.3%). There is no report on essential oil of* A. heterophyllus *leaves, only phytochemical investigation were carried out by using solvents like methanol, ethanol and ethyl acetate extracts.*

Table1. Essential oil composition of A. heterophyllus *leaves*

S.No	Compound name	% composition
1	Cyclohexene,3 methyl-6-(1-methylethylid	0.3
2	Tetradecane,2,6,10-trimethyl-	0.1
3	Tritetracontane	53.5
4	1H-Indene,octahydro-2,3a-trimethyl-2-	0.1
5	decahydro-4,4,8,9,10-pentamethylnapthal	0.8
6	Tetracontane,3,5,24-trimethyl-	16.7
7	3-Methyl-5-(1,4,4-trimethylcyclohex-2-en	0.3
8	2-Hexyl-1-octanol	11.2
9	1-Hentetracontanol	10
10	1-Hexacosene	4.2
11	Octadecane-1-(ethenyloxy)-	2.3
12	E-8-Methyl-9-tetradecen-1-ol acetate	0.1

(ISBN)
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B. Antibacterial activity

Antibacterial activity for the essential oil of A.heterophyllus was carried out against three Gram +ve and three Gram -ve bacteria (Staphylococcus faecalis, S.aureus, Bacillus cereus, Salmonella typhimurium, Shigella flexneri and Vibrio cholera. *The essential oil showed better activity compared to standard Ampicillin.The results indicated that the essential oil inhibited the bacterial strains in dose dependant manner. the Zone of inhibition was given in the table.2*

Table.2.Antibacterial activity of essential oil.

S. No.	Organisms	Essential oil diffusion in mm	Standard in mm
1	*S. faecalis*	4.2	2.7
2	*S. aureus*	4.1	3.4
3	*B. cereus*	5.0	4.1
4	*S.typhimurium*	3.8	4.5
5	*S. flexneri*	5.2	4.0
6	*V. cholerae*	3.9	2.8

***C. In vitro* Antioxidant activity**

1. DPPH free radical scavenging assay of *A.heterophyllus*

The present study examines antioxidant activity of essential oil of *A. heterophyllus* and showed a concentration dependent antiradical activity (Table: 3) by inhibiting DPPH radical with IC_{50} value of 60.17 µg/ml (BHT as the control with IC_{50} value of 18.8 µg/ml). DPPH is a purple colored free radical which on reaction with the plant extract changes to yellow colored stable compound and the extent of the reaction is depends on the hydrogen releasing capacity of the antioxidant. So the essential oil of *A. heterophyllus* has the ability to stop chain reactions of free radicals by forming the stable compounds. Also in the present study, essential oil of *A. heterophyllus* showed better activity in competing with oxygen to react with DPPH radical.

Table3. Antioxidant activity of *A. heterophyllus*

S.NO	Concentration (µg/ml)	Percentage Inhibition	IC_{50} (µg/ml)
1.	25	33.21	60.17
2.	50	45.85	
3.	75	63.22	
4.	100	75.82	
BHA			**18.8**

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IV. Conclusions

Twelve compounds identified from the essential oil obtained from *A.heterophyllus* utilizing GC/MSmethod. Antibacterial activity was tested against six bacterial strains and exhibited better results compared to the standard drug Ampicillin and I*n vitro* antioxidant activity of essential oil also evaluated and showed better activity comparable with Standard BHT. Further studies are required to findout the actual mechanism for biological activities.

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