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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY/MASS SPECTROMETRY ANALYSIS OF *TERMINALIA CATAPPA* L. NUT AND ANTIMICROBIAL ASSAY

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ABSTRACT

Objective: The present study was carried out with the main objective of studying *Terminalia catappa* nut phyto-constituents through GC-MS/MS, as well as its antibacterial, antifungal activities.

Methods: The *T. catappa* nut collected was shade dried and extracted with ethanol, the obtained ethanol extract was used for the whole study- GC-MS/MS, antimicrobial activity.

Results: The results of GC-MS/MS showed 12 peaks. Among the 12 peaks obtained, the highest peak area percent of 48.58 for Propane, 1,1-diethoxy- and 24.36% for t-Butyl hydrogen phthalate followed by 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane showing 12.80 as peak area percent and all the other compound was found to be moderate in peak area percent which was in the range of 3.52-1.10 except β-l-Arabinopyranoside methyl, and Cyclopropanecarboxylic acid, oct-3-en-2-yl ester as its peak area percent was very low showing 0.08 and 0.13. The antibacterial activity was good with *Escherichia coli* when compared to *Staphylococcus aureus* likewise, the antifungal activity was good with *Candida albicans* on comparison with *Aspergillus niger.*

Conclusion: The naturally occurring non –nutrient plant chemicals called as phytochemicals especially phenolics – a natural antioxidant contained in *T. catappa* nut would have contributed for its antibacterial, antifungal properties by interfering with the phospholipid bilayer of the cell membrane and destroying the enzymes required for cellular processes ensuring its therapeutic potential. The variation in therapeutic potential depends on the nutrient content of the soil and other climatic conditions. This confirms the need, validity for phytochemical characterization via analytical methods.

Keywords: Antimicrobial, Analytical, Gas chromatography-mass spectrometry/MS, *Terminalia* sp.

INTRODUCTION

Terminalia catappa is a fast-growing, deciduous, woody tree and grow up to 25 m tall with a trunk of 1-1.5 diameter. It could tolerate strong wind, salt spray, salinity, and grow well in aerated sandy soils. *T. catappa* tree produces fruit from 3 *year*s of age. The nut of *T. catappa* fruit is very tasty similar to almond. Nuts are rich in carbohydrate, moisture, protein, ash, crude fiber, fatty acids, minerals such as sodium, potassium, calcium, magnesium, zinc, vitamins, fiber. The nuts can be eaten raw or by roasting with salt or sugar thus possessing good biological value. In addition to nutrients required for growth, many health benefits are contained in nuts and have antimicrobial, aphrodisiac property. The percentage of nutrient content varies from location to location depending upon the prevailing environmental factors. Since, the nuts are essential and important for general health, we have planned to analyze the phytochemicals through gas chromatography-mass spectrometry (GC-MS/MS) and also its antimicrobial activity with respect to bacteria and fungi.

METHODS

Sample collection

Fresh *T. catappa* nut samples were collected by breaking the shell of *T. catappa* fruit, shade dried, powdered. 25 g of powdered leaf sample was used for ethanol extraction. The extracted sample was used for phytochemical analysis through GC-MS/MS, antimicrobial activity. The plant was authenticated by Dr. A. Balasubramanian. The authentication number was AUT/PUS/070 dated 17/12/2014.

Analytical method

GC-MS/MS was performed on a Scion 436-GC Bruker carrying triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% diphenyl95% dimethylpolysiloxane), 30 m × 0.25 mm ID × 0.25 m df. The column oven temperature program was as follows: 80°C hold for 2 minutes, up to 160°C at the rate of 20°C/minutes - no hold, up to 280°C at the rate of 5°C/minutes - no hold, up to 300°C at the rate of 20°C/minutes - 10 minutes hold, injector temperature 280°C, total GC running time was 41 minutes. The inlet temperature was set at 280°C, source temperature 250°C; ionization mode, ionization at 70-eV ionization energy; For single scan analysis, the scan range was set from m/z 40 to 600; solvent delay: 0-3.5 minutes; and the injection volume was 2 µl. The GC-MS/MS was performed by Institute of Crop Processing Technology, Thanjavur. Here, GC-MS/MS was used as it identifies compounds in complex at trace levels, i.e., lower than the limits of GC-MS.

Antimicrobial assay

The antimicrobial activity was assessed by means of Kirby–Bauer technique [1].

RESULTS AND DISCUSSION

Table 1 depicts the results of compounds identified in *T. catappa* nut.

Totally, 12 compounds were identified when T. catappa nut was analyzed through GC-MS/MS. The compound showing highest peak was propane, 1,1-diethoxy-, t-butyl hydrogen phthalate, 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane. The compound showing peak at moderate level was tert-butyl 2-aminophenylcarbamate ditms, 1H-pyrazole, 4,5-dihydro-3-methyl-1-propyl-, pentanoic acid, cholane-5,20(22)-diene-3b-phenoxy, stigmastan-3,5-diene. The peak was very low for 8-methyloctahydrocoumarin, cyclopropanecarboxylic acid, oct-3-en-2-yl ester, β-l-arabinopyranoside, and methyl. The molecular weight of the compounds range from 102 to 418. *T. catappa* fruit, flesh, nut, shell

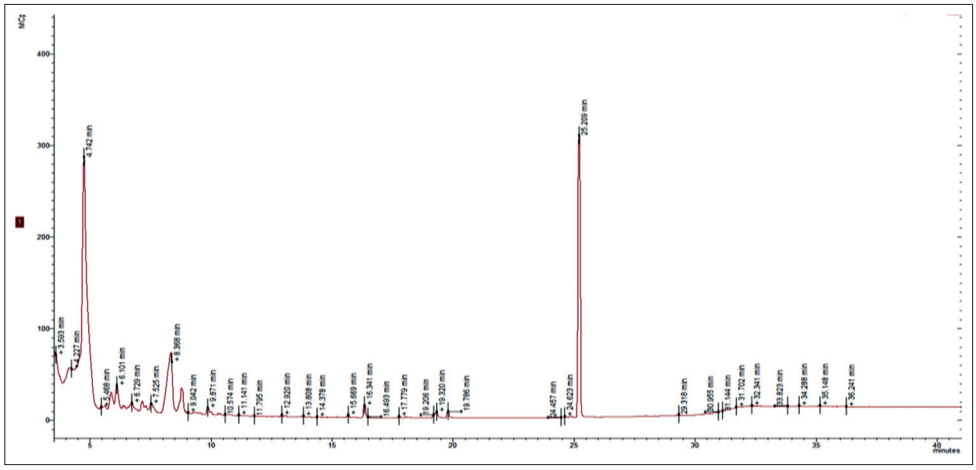

Fig. 1: Chromatogram

Table 1: Components in ethanol extract of *Terminalia catappa* Nut

S.No.	RT	Name of the compound	MF	MW	PA%
1	4.74	Propane, 1,1-diethoxy-	$C_7H_{16}O_2$	132	48.58
2	6.10	1H-Pyrazole, 4,5-dihydro-3-methyl-1-propyl-	$C_7H_{14}N_2$	126	2.93
3	8.36	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	576	12.80
4	8.72	Pentanoic acid	$C_5H_{10}O_2$	102	2.87
5	9.91	β-l-Arabinopyranoside, methyl	$C_6H_{12}O_5$	164	0.08
6	16.34	Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$	228	1.10
7	19.32	8-Methyloctahydrocoumarin	$C_{10}H_{16}O_2$	168	0.81
8	25.20	t-Butyl hydrogen phthalate	$C_{12}H_{14}O_4$	222	24.36
9	29.31	Cyclopropanecarboxylic acid, oct-3-en-2-yl ester	$C_{12}H_{20}O_2$	196	0.13
10	30.95	tert-Butyl 2-aminophenylcarbamate ditms	$C_{17}H_{32}N_2O_2Si_2$	352	3.52
11	32.34	Stigmastan-3,5-diene	$C_{29}H_{48}$	396	1.30
12	35.14	Cholane-5,20 (22)-diene-3b-phenoxy	$C_{30}H_{42}O$	418	1.53

MF: Molecular formulae, MW: Molecular weight, PA: Peak area percent

Table 2: Antimicrobial activity of *Terminalia catappa* Nut

Plant part used	Microbes tested	Zone of inhibition (mm)
Terminalia catappa Nut.	*Escherichia coli*	7
	Staphylococcus aureus	6.5
	Aspergillus niger	11
	Candida albicans	18

was studied by Krishnaveni *et al*. for its phytonutrient, antioxidant, qualitative assay as well as GC-MS/MS analysis, air pollution tolerance index of *T. catappa* leaves [2-7]. Likewise, air pollution tolerance index, antioxidant activity of *Terminalia tomentosa* was studied by Krishnaveni *et al*. [8,9].

The Fig. 1 shows the chromatogram obtained for the phytochemicals studied through GC-MS/MS.

Antimicrobial assay

Table 2 depicts the antimicrobial activity of ethanol extract of *T. catappa* nut.

The *T. catappa* nut was found to act against both bacteria and fungi. The zone of inhibition observed for bacteria like *Escherichia coli* was 7 mm and for *Staphylococcus aureus* it was 6.5 mm. The zone of inhibition observed for fungi was 11 *mm* for *Aspergillus niger* and 18 mm for *Candida albicans*. The antimicrobial activity was higher for fungi when compared to bacteria. The obtained results predicts the ability to kill the microbes which was confirmed through their zone of inhibition.

CONCLUSION

The *T. catappa* nut possess good nutritional, biological, therapeutic value. The nuts are offering numerous health benefits, the therapeutic property of nut was mainly attributed by its phytochemicals. The phytochemicals present in the *T. catappa* nut contribute for its antifungal, antibacterial properties, which find application in pharmaceutical industry. Apart from this property, it has to be further studied for the individual property in all aspects in order to open different avenues.

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