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Bioactive compounds in ethanolic extract of *Strychnos innocua* root using gas chromatography and mass spectrometry (GC-MS)

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Abstract
Medicinal plants are of great relevance with endless pharmaceutical and therapeutic properties. They are source of wide array of secondary metabolites or bioactive compounds/phytochemicals (phenols, alkaloids, flavonoids, terpenoids, tannins, steroids and saponins) which are capable of discharging numerous biological functions (antimicrobial, antioxidant, antiviral, antifungal, anti-fibrotic, immune-modulatory, cytotoxic, antipyretic, antitumor, antihelminthic, antiprotozoal, antibacterial and so on). The use of gas chromatography and mass spectrometry analysis in identifying the bioactive compounds in ethanolic extract of *Strychnos innocua* root is a fundamental technique in quantifying the unknown samples, trace elements and contaminants leading to the discovery of novel compounds of pharmaceutical and biomedical importance. Result obtained showed that *Strychnos innocua* root extract contains 39 bioactive compounds with α-Cubebene (20.09%) having the highest concentration followed by Dibutyl benzene-1,2–dicarboxylate (10.17%), β-Elemenone (10.02%), 4-Methoxy-2-nitroformanilide (7.21%), 1-Methyl cyclopropane methanol (5.96%), 1, 3 propanediol, 2-ethyl 2-hydroxymethyl (3.71%), Azelaic acid (2.87%), Glycidol stearate (2.85%), Chloromethyl 2-chlorodecanoate (2.83%) and γ-terpinene (2.56%) respectively. The remaining 29 bioactive compounds have concentrations less than 2%. It was concluded that all the compounds observed are sources of medication that can be used traditionally in the treatment of human and animal diseases.

Keywords: *Strychnos innocua*, phytochemicals, pharmaceuticals, therapeutics, gas chromatography

Introduction

Medicinal plants are the major components of almost all indigenous or alternative systems of medicines. They contain phytochemicals which are safe, non-toxic and easily affordable (Singh *et al*., 2022; Shittu and Alagbe, 2020) [9, 15]. According to WHO (1996) [39] about 80% of the population in developing countries rely on medicinal plants for the treatment of various ailments. There are over 2000 medicinal plants with high potential that are yet to be explored (Oluwafemi *et al*., 2020) [12]. Bioactive compounds from these medicinal plants can perform an anti-inflammatory, antifungal, antiviral, antioxidant, immune-stimulatory, analgesics, antibacterial, anti-proliferative, cytotoxic and hepato-protective, antipyretic, antihelminthic, antiprotozoal, anti-depressant, anti-tumor, anti-fibrotic and hypolipidemic properties (Alagbe, 2022; Olafadehan *et al*., 2021; Agubosi *et al*., 2022) [4, 16, 10-11] and could also aid in the discovery of drugs (Vasquez *et al*., 2017; Hirotani *et al*., 1991; Muritala *et al*., 2022) [7, 29, 3].

Strychnos innocua also known as Natal orange belongs to the family Loganiaceae, genus Strychnos and order Gentianales. The tree is found in several countries such as; Angola, Guinea, Madagascar, Malawi, South Africa, Sudan, Mali, Uganda, Malawi, Zimbabwe, Zambia, Ethiopia and some parts of India (Maghembe, 1994) [41]. The trees are found in riverine fringes, sand forest and it can grow up to 3-14 m high with simple leaves characterized by rounded emarginate or subacute apex (Hines and Eckman, 1993) [40]. Extracts from the leaves, roots and stems can be used traditionally for the treatment of snake bites, gastrointestinal, skin diseases, pneumonia and sexually transmitted infections (Al-Wathnani, 2012) [37].

Previous studies have revealed that *Strychnos innocua* leaf, stem and root extract contains several bioactive compounds with antimicrobial properties and are also capable of inhibiting the activity of some bacteria and fungi such as; *Bacillus* spp, *Candida* spp, *Alternaria solani*, *Brevibacillus brevis*, *Cochliobolus lunatus*, *Escherichia coli*, *Enterobacter* spp, *Aspergillus* spp, *Fusarium* spp, *Klebsiella* spp, *Monascus ruber*, *Micrococcus luteus*, *Pseudomonas* spp, *Streptococcus* spp, *Styphylococcus* spp, *Salmonella* spp and *Shigella shiga*. Phytochemical analysis of methanolic extract from leaves and roots of *Strychnos innocua* revealed the presence of phenolic compounds which are capable of scavenging free radicals (antioxidants) (Hamisu *et al*., 2021; Lee *et al*., 2011; Igbal *et al*., 2011) [36, 23, 25], preventing the risk of cardiovascular disease (Alagbe *et al*., 2022; Alagbe, 2021) [4, 18] and performing immune-modulatory activities in animals thus encouraging food safety (Oloruntola *et al*., 2018; Halliwell and Gutteridge, 1998) [2, 1]. The aim of this study was to evaluate the bioactive compounds in ethanolic extract of *Strychnos innocua* root using gas chromatography and mass spectrometry (GC-MS).

Materials and Methods

Experimental site, collection and preparation of *Strychnos innocua* ethanolic leaf and root extract

The study was performed at Sumitra Research Institute, Gujarat, India with a coastline of 1,600 Km, 23º 13'N 72°41'E. Fresh *Strychnos innocua* root was harvested from Waghai village, Saputara, India and identified at the Department of Biological Sciences, Sumitra Research Institute, Gujarat, India. The harvested roots was washed with distilled water, dried under the shade for 13 days and blended into powder form with the aid of electric blender and kept in an air tight labeled container. 100 g of grinded sample was soaked in 350 mL of 90% ethanol for 48 hours with occasional stirring thereafter samples was sieved using Whatman's No. 1 filter paper (10 cm) and stored in a sterile air tight container and stored in a cool dry place until further use.

Gas chromatography and mass spectrometry (GC-MS) of ethanolic extract from *Strychnos innocua* root

Gas chromatography mass spectrometry (GC-MS) analysis of ethanolic extract from *Strychnos innocua* root was performed with a Varian 450 GC system (Model 1842 series, China) equipped with fused silica column and it was operated at a temperature and pressure range of 50 °C to 450 °C isothermal 1079 PTV injector and 0 to 100 psi, consisting of splitless injector with total flow of 500 mL/minutes at 10 psi, electron range of 150eV. Secondary compounds were identified with standard compounds in National Institute of Standard and Technology (NIST).

Table 1: Secondary metabolites in *Strychnos innocua* ethanolic root extract using GC-MS

Bioactive compounds	Area (%)	R.T (min)	Functions
Di-ethyl suberate	1.44	1.450	Antimicrobial and antioxidant
Ethyl Oleate	0.72	1.931	Antipyretic and antioxidant
Diisooctyl phthalate	0.01	2.500	Anti-depressant and antifungal
Glycidol stearate	2.85	3.444	Anitiviral, hepato-protective and antioxidant
1,2 – Benzenedicarboxylic acid	1.77		Anti-microbial, anti-proliferative
Monomethyl pimelate	0.02	6.091	Antifungal
γ-terpinene	1.10	9.435	Hepatoprotective and antifungal
4-fluoro-1-methyl-5-carboxylic acid	0.40	10.701	Anti-inflammatory, antibacterial and analgesics
3-Allyl-6-methoxyphenol	1.67	11.331	Antiprotozoal and cytotoxic
Cyclooctane	0.10	15.560	Anti-androgenic, antiviral and anti-inflammatory
Formamide	2.05	15.740	Hepato-protective, hypolipidemic, antimicrobial and antioxidant
α-cubebene	20.09	15.100	Antibacterial, antifungal, angelsics antipyretic and antioxidant
2,4,6 –Octatrien-1-ol	0.77	15.607	Antiviral and antioxidant
9,12-Octadecanoic acid	1.06	18.351	Cytotoxic, antioxidant, anti-inflammatory, antitumor, antifungal
α-longipinene	0.15	18.220	Anti-inflammatory, antioxidant, anti-depressant and antifungal
Azelaic acid	2.87	18.306	Anti-fibrotic, anti-inflammatory and hypolipidemic
Terpinen-4-ol	1.51	18.331	
1,3 propanediol, 2-ethyl 2-hydroxymethyl	3.71	18.211	Antibacterial, anti-inflammatory, antipyretic, antihelminthic and antifungal
γ-Terpinene	2.56	19.386	Amtioxidant and anti-inflammatory
β-Elemenone	10.02	19.931	Cytotoxic and hepato-protective
9-Octadecenoic acid	1.16	19.510	Antifungal
Torreyol-α-cadinol	0.83	19.259	Anitiviral, hepato-protective and antioxidant
Hepatadec-3-enal	0.30	19.400	Anti-microbial, anti-proliferative, antiviral, antihelminthic and antibacterial
Ethylene diacrylate	0.50	20.209	Analgesics, antibacterial, antifungal
1-Hexyl -2 nitrocyclohexane	1.62	21.344	Anti-inflammatory, antioxidant, anti-depressant
Chloromethyl 2-chlorodecanoate	2.83	21.381	Anti-fibrotic, anti-inflammatory
5-methylhexan-2-yl hepadecyl benzene -1,2 dicarboxylate	1.10	21.100	Antifungal, angelsics antipyretic and antioxidant
1-Methyl cyclopropane methanol	5.96	22.891	Hepatoprotective and antifungal
4-Acetoxy-3-methoxystyrene	1.14	23.080	Hepato-protective, hypolipidemic, antimicrobial
4-Methoxy-2-nitroformanilide	7.21	23.300	Cytotoxic, antioxidant
α-Terpinolene	0.02	23.701	Antioxidant, anti-proliferative
3-deoxy-d-mannoic acid	1.20	25.186	Antioxidant, anti-proliferative, antifungal and anti-inflammatory
Dibutyl benzene -1,2 - dicarboxylate	10.17	25.901	Cytotoxic and hepato-protective

Butyl undecyl benzene-1,2- dicarboxylate	1.31	25.670	Cytotoxic, antioxidant, anti-inflammatory, antitumor, antifungal
9,15 – Octadecadienoic acid	0.06	26.801	Antibacterial, antifungal, angelsics antipyretic
β-Cyclocitral	0.01	28.009	antitumor, antifungal
α-Phellandrene	1.10	34.491	Angelsics antipyretic and antioxidant
β-Phenethylamine	0.08	38.567	Anti-bacterial
Humulene	1.12	41.340	Antimicrobial, antifungal and hypolipidemic
Total	92.59		

R.T: reaction time (minutes)

Results and Discussion

Medicinal plants contain natural compounds or phytochemicals that are eco-friendly, safe and locally available with pharmacological properties (Musa *et al*., 2020; Adewale *et al*., 2021) [14, 13]. They can also be used traditionally for the treatment of various ailments such as cold, cough, gastrointestinal disease, skin disease, respiratory disease, malaria, typhoid and snake bites (Nascimento *et al*., 2000) [42]. Bioactive compounds are mostly secondary metabolites produced by plants via subsidiary pathways and are used by plants for growth, or defense against pathogens (Okeke *et al*., 2001; Oluwafemi *et al*., 2021; Narayani *et al*., 2012) [43, 45, 12]. Secondary metabolites in *Strychnos innocua* ethanolic root extract using gas chromatography and mass spectrometry (GC-MS) (Table 1) reveals that it is largely contains α-Cubebene (20.09%), Dibutyl benzene -1,2 – dicarboxylate (10.17%), β-Elemenone (10.02%), 4-Methoxy-2-nitroformanilide (7.21%), 1-Methyl cyclopropane methanol (5.96%), 1,3 propanediol, 2-ethyl 2-hydroxymethyl (3.71%), Azelaic acid (2.87%), Glycidol stearate (2.85%), Chloromethyl 2-chlorodecanoate (2.83%) and γ-terpinene (2.56%) respectively. Other compounds reported were less than 2.0% however, they all have a marked therapeutic functions (anti-inflammatory, antiviral, antifungal, antioxidant, hypolipidemic, angelsics, anti-pyretic, cytotoxic, antitumor and anti-depressant activities) (Okeke *et al*.,) [43]. The GC-MS component analyzed in this study is in consonance with the findings of Hamisu *et al*. (2021) [36] but contrary to the reports of Hoet *et al*. (2006) [30]. These dissimilarity can be ascribed to processing or extraction procedures employed, parts of plant used, species, geographical location, age of plant as well as method of harvesting (Omokore and Alagbe, 2019; Hoet *et al*., 2006) [17, 30]. The presence of phytochemicals in *Strychnos innocua* ethanolic root extract reveals that it has the ability to scavenge toxic chemicals in the body, inhibit the activities of pathogenic bacteria in the gut of animals, thus enhancing the absorption of nutrient as well as enhances the activities of enzymes (Oluwafemi *et al*., 2021; Narayani *et al*., 2012) [12, 45].

Conclusion

Medicinal plants have so several health benefits due to the presence of phytochemicals (alkaloids, flavonoids, phenols, terpenoids, saponins, tannins and steroids). Analyzing the bioactive components in *Strychnos innocua* ethanolic root extract will unleash some of the potential pharmaceutical properties in the plant. Adopting the use of gas chromatography and mass spectrometry will further aid in identifying and quantifying unknown samples, unknown contaminants, trace elements and gases.

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