

# 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  $\alpha$ -Cubebene (20.09 %) having the highest concentration followed by Dibutyl benzene -1,2 – dicarboxylate (10.17 %),  $\beta$ -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  $\gamma$ -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

**1. 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). According to WHO, (1996) 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). 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) and could also aid in the discovery of drugs (Vasquez et al., 2017; Hirotani et al., 1991; Muritala et al., 2022).

*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). 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). 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 (Hongxing et al., 2005; Al-Wathnani, 2012).

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* (Iyun et al., 2022; Sallau et al., 2022). 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), preventing the risk of cardiovascular disease (Alagbe et al., 2022; Alagbe, 2021) and performing immune-modulatory activities in animals thus encouraging food safety (Oloruntola et al., 2018; Halliwell and Gutteridge, 1998). 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).

## 2. 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, 23° 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 were 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 split less 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).

## 3. RESULTS & DISCUSSION

**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	Anitviral, 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
$\alpha$ -cubebene	20.09	15.100	Antibacterial, antifungal, analgesics 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
$\alpha$ -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
$\gamma$ -Terpinene	2.56	19.386	Antioxidant and anti-inflammatory
$\beta$ -Elemenone	10.02	19.931	Cytotoxic and hepato-protective
9-Octadecenoic acid	1.16	19.510	Antifungal
Torreyol- $\alpha$ -cadinol	0.83	19.259	Antiviral, 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 heptadecyl benzene -1,2 dicarboxylate	1.10	21.100	Antifungal, analgesics 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
$\alpha$ -Terpinolene	0.02	23.701	Antioxidant, anti-proliferative
3-deoxy-d-mannonic 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, analgesics antipyretic
$\beta$ -Cyclocitral	0.01	28.009	antitumor, antifungal
$\alpha$ -Phellandrene	1.10	34.491	Analgesics antipyretic and antioxidant
$\beta$ -Phenethylamine	0.08	38.567	Anti-bacterial
Humulene	1.12	41.340	Antimicrobial, antifungal and hypolipidemic
Total	92.59		

R.T: reaction time (minutes)

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). 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; Perekh et al., 2007). 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). Secondary metabolites in *Strychnos innocua* ethanolic root extract using gas chromatography and mass spectrometry (GC-MS) (Table 1) reveals that it is largely contains  $\alpha$ -Cubebene (20.09 %), Dibutyl benzene -1,2 – dicarboxylate (10.17 %),  $\beta$ -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  $\gamma$ -terpinene (2.56 %) respectively. Other compounds reported were less than 2.0 % however, they all have a marked therapeutic function (anti-inflammatory, antiviral, antifungal, antioxidant, hypolipidemic, angelsics, anti-pyretic, cytotoxic, antitumor and anti-depressant activities) (Okeke et al., 2001; Tease and Evans, 1989). The GC-MS component analyzed in this study is in consonance with the findings of Hamisu et al., (2021) but contrary to the reports of Hoet et al., (2006). This 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). 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).

#### 4. 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.

#### Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for species collection, identification & experimentation.

#### Informed consent

Not applicable.

#### Conflicts of interests

The authors declare that there are no conflicts of interests.

#### Funding

The study has not received any external funding.

#### Data and materials availability

All data associated with this study are present in the paper.

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