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ORIGINAL ARTICLE

# Phytochemical Screening and Gas Chromatography-Mass Spectrometry Analysis of Ethanol Extract of *Scambiosa columbabria* L.

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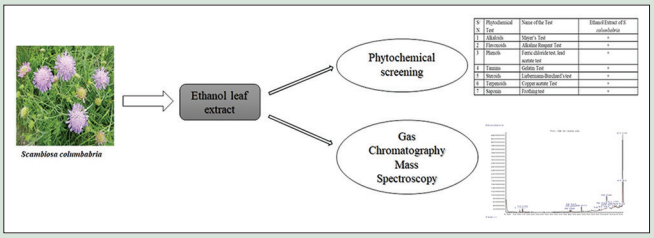
## ABSTRACT

**Background:** *Scambiosa columbabria* L. is commonly known as the wild scabious and is used in South Africa to treat various ailments such as wound bruises, painful menstruation, heartburn, and colic. Despite its extensive traditional usage, the bioactive constituents of this plant are yet to be explored. **Objective:** The present study was carried out to identify the bioactive constituents of the ethanol extract of *S. columbabria* by phytochemical screening and gas chromatography-mass spectrometry (GC-MS). **Materials and Methods:** Sixty grams of the powdered leaves was sequentially extracted by ethanol and later tested for preliminary phytochemical screening and further subjected to GC-MS analysis. **Results:** The results showed the presence of alkaloids, flavonoids, phenolics, tannins, steroids, terpenoids, and saponins in the extract. The GC-MS analysis revealed the presence of 16 major compounds, with flavonoids (40.84%) being the most represented chemical class. **Conclusion:** The findings indicated that the plant possesses compounds with biological activities and therefore justifies its traditional usage in the treatment of skin and other diseases.

**Key words:** Biological activity, diseases, gas chromatography-mass spectrometry, phytochemical screening, *Scambiosa columbabria*

## SUMMARY

*Scambiosa columbabria* L. is commonly known as the wild scabious and is used in South Africa to treat various ailments such as wound bruises, painful menstruation, heartburn, and colic. The result generated from the study reveals the presence of various bioactive compounds which are known to exhibit various biological activities and therefore validates the reports of therapeutic importance of this plant in the treatment of skin and other diseases, and it may therefore be recommended as plant of pharmaceutical importance.




**Abbreviations Used:** GC-MS: Gas chromatography-mass spectrometry, MSD: Mass selective detector, WHO: World Health Organization.

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## INTRODUCTION

Recently, the use of plant products in ethnomedicine is increasing globally. The World Health Organization has also pointed out that about 80% of the world's population rely heavily on herbal medicinal products for the treatment of various health challenges.[1,2] These products are being prepared as decoction, infusions, extracts, or pastes with easily accessible liquids such as alcohol, water, and milk. In South Africa, the use of herbs to treat various illnesses continues to expand rapidly across all provinces, and studies have also indicated that South Africa hosts a variety of about 30,000 plant species which are used for treating numerous ailments such as skin, cancer, diabetes, tuberculosis, and hypertension.[3] However, the bioactive constituents responsible for the therapeutic activity of many South African medicinal plants have not been scientifically elucidated, and such is the case of *Scambiosa columbabria* which is investigated in the current study.

*S. columbabria* L. is a perennial herb that grows to about 1 m in height, with annual branches developing from persistent fleshy roots.[4] It belongs to the family Caprifloliaceae (formerly Dipasacaceae). The plant is locally called wild scabious (English); bitterwortel (Afrikaans); makgha (Xhosa); ibheka (Zulu); and moholungoane (South Sotho). *S. columbaria* occurs mostly in grasslands and rocky slopes, is widely distributed throughout South Africa, and is particularly common in the Western Cape. Conventionally, the dried roasted roots of the plant are made into a wound-healing ointment to treat wound bruises, and the roots are used to prepare a pleasant smelling baby powder.[5] In addition, the root and leaves are also used to treat painful menstruation, heartburn, and colic. Despite the extensive traditional usage of this plant, to date, no published *in-vitro* or *in-vivo* studies are available in literature. Some of the compounds previously identified from the rootstock of *S. columbabria* include loganin and sweroside.[4] However, there is still a lack of comprehensive studies with regard to the active constituents of this plant. Hence, the present study was carried out to identify the bioactive constituents of the ethanol extract of *S. columbabria* by phytochemical screening and gas



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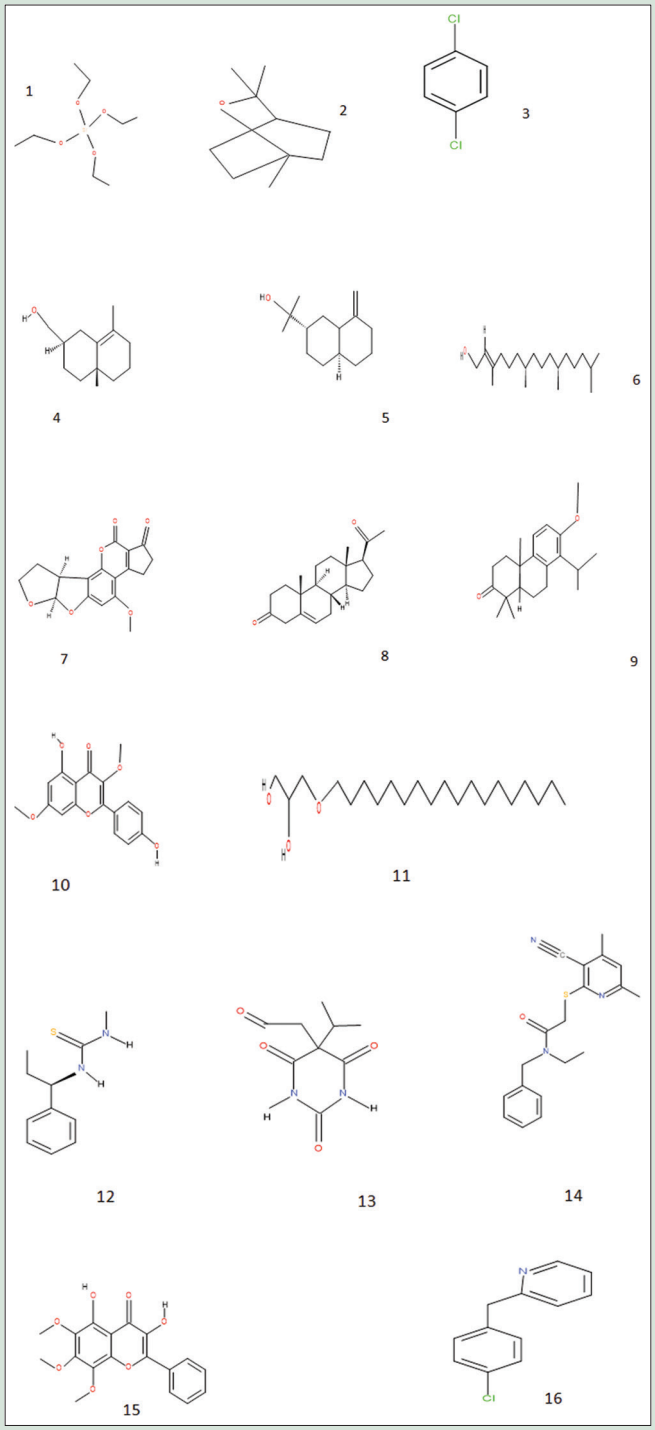


**Figure 1:** Chemical structure of identified compounds from *Scambiosa columbabria*. Tetraethyl silicate (1) Eucalyptol (2), Benzene, 1,4-dichloro- (3), 2-Naphthalenemethanol, 1,2,3,4,4a, 5,6,7-octahydro-.alpha., alpha., 4a, 8-tetramethyl-, (2R-cis)- (4), 2-Naphthalenemethanol, decahydro-α, α,4a-trimethyl-8-methylene-, [2R-(2α,4aα,8aβ)]- (5), Phytol (6), Aflatoxin B2 (7), Pregn-5-ene-3,20-dione (8), Podocarpa-8,11,13-trien-3-one, 14-isopropyl-13-methoxy-(9),4H-1-Benzopyran-4-one,5-hydroxy-2-(4-hydroxyphenyl)-3,7-dimethoxy-(10), Batilol(11),1-Methyl-3-[(1R)-1-phenylpropyl] thiourea(12),Aprobarbital(13),Acetamide,N-benzyl-2-(3-cyano-4,6-dimethylpyridin-2-ylsulfanyl)-N-ethyl-(14), 3,5-Dihydroxy-6,7,8-trimethoxyflavone(15), 4-(para-Chlorobenzyl)-pyridine (16)

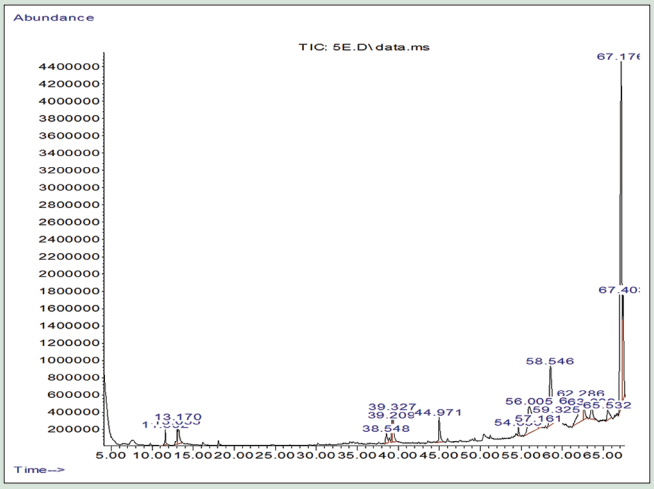


**Figure 2:** Gas chromatography-mass spectrometry chromatogram of ethanolic extract of *Scambiosa columbabria* leaves

**Table 1:** Qualitative screening of phytochemical components present in the ethanol extract of *Scambiosa columbabria*

| Phytochemical test | Name of the test | Ethanol extract of *Scambiosa columbabria* |
|---|---|---|
| Alkaloids | Mayer's test | + |
| Flavonoids | Alkaline reagent test | + |
| Phenols | Ferric chloride test, lead acetate test | + |
| Tannins | Gelatin test | + |
| Steroids | Liebermann-burchard's test | + |
| Terpenoids | Copper acetate test | + |
| Saponin | Frothing test | + |

+: Present

chromatography-mass spectrometry (GC-MS) with the view to justify its ethnobotanical usage.

## MATERIALS AND METHODS

### Plant collection

The whole plant material together with the leaves of *S. columbabria* was collected from Alice, Eastern Cape province of South Africa, during the month of January, 2019, at 10:00 AM. The collected plant was authenticated by a botanist at the Giffen Herbarium of the University of Fort Hare, where voucher specimens are kept. The leaves of *S. columbabria* were detached from the rest of the plant after which they were washed with clean tap water thrice to eradicate debris and later oven-dried to a constant weight at 30°C for 1 week. Thereafter, the dried leaves were then milled to a homogenous powder using an electric blender (Polymix PX-MFC 90D, KINEMATICA AG, Luzern, Switzerland).

### Plant extraction

About 60 g of the powdered leaves was extracted in 1000 ml of ethanol (99.99%) and placed on an orbital shaker (Labcon laboratory service [Pty], South Africa) for 24 h. The extract was later filtered using a Buchner funnel and Whatman No. 1 filter paper. The resulting filtrate was concentrated to dryness using a rotary evaporator (Heidolph Laborata 4000, Heidolph instruments, GmbH and Co, Germany). The final extract was weighed to determine the yield (4.54%) and then stored at 4°C in a refrigerator for further studies.

**Table 2:** The major phytocompounds detected in the ethanolic leaf extract of *Scambiosa columbabria* by gas chromatography-mass spectrometry analysis

| Retention time (min) | Compound name | Molecular formular | Molecular weight | Area (%) |
|---|---|---|---|---|
| 11.602 | Tetraethyl silicate | $C_8H_{20}O_4Si$ | 208 | 0.79 |
| 13.055 | Eucalyptol | $C_{10}H_{18}O$ | 154 | 1.11 |
| 13.17 | Benzene, 1,4-dichloro- | $C_6H_4Cl_2$ | 147 | 2.24 |
| 38.548 | 2-Naphthalenemethanol, 1,2,3,4,4a, 5,6,7-octahydro-.alpha.,.alpha.,4a, 8-tetramethyl-, (2R-cis)- | $C_{15}H_{26}O$ | 222 | 4.45 |
| 39.209 | 2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8-methylene-, [2R-(2α,4aα,8aβ)]- | $C_{15}H_{26}O$ | 222 | 3.66 |
| 54.639 | Phytol | $C_{20}H_{40}O$ | 296 | 0.54 |
| 56.005 | Aflatoxin B2 | $C_{17}H_{14}O_6$ | 314 | 10.45 |
| 57.161 | Pregn-5-ene-3,20-dione | $C_{21}H_{30}O_2$ | 314 | 0.35 |
| 58.546 | Podocarpa-8,11,13-trien-3-one, 14-isopropyl-13-methoxy- | $C_{21}H_{30}$ | 314 | 13.71 |
| 59.325 | 4H-1-Benzopyran-4-one, 5-hydroxy-2-(4-hydroxyphenyl)-3,7-dimethoxy- | $C_{17}H_{14}O_6$ | 314 | 0.68 |
| 62.286 | Batilol | $C_{21}H_{44}O_3$ | 344 | 6.86 |
| 62.592 | 1-Methyl-3-[(1R)-1-phenylpropyl] thiourea | $C_{11}H_{16}N_2S$ | 208 | 1.71 |
| 63.602 | Aprobarbital | $C_{10}H_{14}N_2O_3$ | 210 | 2.1 |
| 65.532 | Acetamide, N-benzyl-2-(3-cyano-4,6-dimethylpyridin-2-ylsulfanyl)-N-ethyl- | $C_{19}H_{21}N_3OS$ | 339 | 1.73 |
| 67.176 | 3,5-Dihydroxy-6,7,8-trimethoxyflavone | $C_{18}H_{16}O_7$ | 344 | 40.84 |
| 67.403 | 4-(para-Chlorobenzyl)-pyridine | $C_{12}H_{10}CIN$ | 203 | 8.78 |
| Total identified components (%) | | | | 100 |

**Table 3:** Nature of the compounds identified in the extract of *Scambiosa columbabria*

| Compound name | Nature of compound |
|---|---|
| Tetraethyl silicate | Ester |
| Eucalyptol | Monoterpenoid |
| Benzene, 1,4-dichloro- | Phenol |
| 2-Naphthalenemethanol, 1,2,3,4,4a, 5,6,7-octahydro-.alpha.,.alpha.,4a, 8-tetramethyl-, (2R-cis)- | NA |
| 2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8-methylene-, [2R-(2α,4aα,8aβ)]- | NA |
| Phytol | Diterpenes |
| Aflatoxin B2 | Coumarins |
| Pregn-5-ene-3,20-dione | Steroid |
| Podocarpa-8,11,13-trien-3-one, 14-isopropyl-13-methoxy- | Steroid |
| 4H-1-Benzopyran-4-one, 5-hydroxy-2-(4-hydroxyphenyl)-3,7-dimethoxy- | NA |
| Batilol | Alkyl glycerol |
| 1-Methyl-3-[(1R)-1-phenylpropyl] thiourea | NA |
| Aprobarbital | Diazine |
| Acetamide, N-benzyl-2-(3-cyano-4,6-dimethylpyridin-2-ylsulfanyl)-N-ethyl- | NA |
| 3,5-Dihydroxy-6,7,8-trimethoxyflavone | Flavonoid |
| 4-(para-Chlorobenzyl)-pyridine | Pyridine |

N/A: Not available in database

**Table 4:** Class composition of the compounds identified in the extract of *Scambiosa columbabria*

| Class of compound | *Scambiosa columbabria* |
|---|---|
| Alkyl glycerol | 6.86 |
| Coumarins | 10.45 |
| Diterpenes | 0.54 |
| Diazines | 2.1 |
| Esters | 0.79 |
| Flavonoids | 40.84 |
| Monoterpenes | 1.11 |
| Phenols | 2.24 |
| Steroids | 14.06 |
| Others | 21.01 |
| Total grouped components (%) | 100 |

**Table 5:** Reported biological activity of some compounds identified in the extract of *Scambiosa columbabria*

| Name of compound | Reported biological activity | Reference |
|---|---|---|
| Eucalyptol | Anti-inflammatory, antioxidant and antibacterial | [37,39,40] |
| Benzene, 1,4-dichloro- | Deodorant | [32] |
| Phytol | Cytotoxic, antinociceptive, antioxidant, antimicrobial, anti-inflammatory, anticancer, diuretic | [37,41] |
| Pregn-5-ene-3,20-dione | Antibacterial and antilarva | [36] |
| Batilol | It is used in cosmetics as a stabilising ingredient and skin-conditioning agent | [37] |
| Aprobarbital | anticonvulsant properties and used for the treatment of insomnia | [32] |
| 3,5-Dihydroxy-6,7,8-trimethoxyflavone | Antifugal | [38] |

### Phytochemical screening

The presence or absence of phytochemical constituents such as alkaloids, flavonoids, phenolics, tannins, steroids, terpenoids, and saponins was screened using standard qualitative procedures.[6-8]

### Gas chromatography-mass spectrometry analysis

The GC-MS analysis of the ethanol extract of *S. columbabria* was carried out using an Agilent 7890 GC system equipped with an Agilent 5977A Mass selective detector system (Chemetrix [Pty] Ltd., Agilent Technologies, DE, Germany) with a Zebron-5MS (cross-linked 5% – phenyl methyl polysiloxane) column (HP-5 fused silica 30 m × 0.320 mm × 0.250 µm film thickness). The instrument was operated at injection temperature (250°C), ion source temperature (280°C), and pressure (48.745 kpa). The carrier gas used was GC-grade helium (99.999% purity) at a flow rate of 36.262 cm/s, and about 1 µL sample injections was used. The oven temperature was initially started from 40°C (held for 1 min) and then ramped to 240°C at 3°C/min.

### Identification of components

Identification of components was conducted using the National Institute of Standard and Technology 11 library source. The mass spectrum of individual unknown compound was achieved by comparing their retention times with those of the known compound in the software database library.

## RESULTS AND DISCUSSION

To investigate the importance of any medicinal plant, the initial or first step is to screen for its phytochemicals, as it gives a broad knowledge with respect to the nature of the compounds present in it. In the present study, the ethanolic leaf extracts of *S. columbabria* were preliminarily screened for the phytochemicals.

The extract shows the presence of alkaloids, flavonoids, phenolics, tannins, steroids, terpenoids, and saponins [Table 1]. These observed phytochemicals have been reported to possess a broad range of activities.[9-11] Alkaloids present in the extract of *S. columbabria* have been reported to possess anticonvulsant,[12] hypotensive,[13] antimicrobial,[12] antiprotozoal,[14] and antimalarial activities.[15] In addition, studies have also shown that plants with alkaloids may have hypoglycemic effect via insulin-releasing mechanism and insulin-mimicking activity, thus improving postprandial hyperglycemia.[9] The presence of flavonoids in plant extract has also been reported to play an important role in plant biochemistry and physiology as enzyme inhibitor, antioxidants,[16] anti-diabetic,[17-19] anti-inflammatory,[20] antimicrobial,[21-23] antifungal,[21] and anticancer activities, thereby protecting the cells against all stages of carcinogenesis.[10] Furthermore, available report on phenolic compounds has also been demonstrated for their usefulness to prevent oxidative damage in cells, thereby acting as a free radical terminator.[24] Tanins, steroids, and terpenoids present in the extract have been responsible for anti-inflammatory, antioxidant, antibacterial, and hypoglycemic activities.[25,26] It can be observed from this study that the presence of alkaloids, flavonoids, phenolics, tannins, steroids, terpenoids, and saponins in the extract of *S. columbabria* leaves is in support with those reported from previous studies, indicating the presence of these phytochemicals in the plant extracts.[27-29] Therefore, these phytochemicals observed in the ethanol leaf extract of *S. columbabria* are an indication that this plant plays a vital role as a dietary antioxidant, and it can be used to develop new compounds for health benefit.

The results of the GC-MS analysis of *S. columbabria* revealed the presence of sixteen compounds [Figures 1 and 2], representing 100% of the total identified components [Table 2]. The detected compounds class were mainly composed of alkyl glycerol, coumarins, diterpenes, esters, flavonoids, monoterpenes, steroids, phenols, and others [Table 3]. The most represented chemical class were flavonoids (40.84%), steroids (14.06%), coumarins (10.45%), alkyl glycerols (6.86%), phenols (2.24%), monoterpenes (1.11%), ester (0.79%), and diterpenes (0.54%) [Table 4]. However, studies have indicated that compounds mirror the biological activities of plant from which it is extracted. Among the compounds identified, eucalyptol which is a monoterpene found in the extract of *S. columbabria* was reported to control asthma via anti-inflammatory cytokine inhibition [Table 5].[30] In addition to anti-inflammatory activity, eucalyptol was also reported to be frequently used in the manufacturing of cosmetics, thereby increasing the percutaneous penetration of drugs as aromatherapy agent.[31] Benzene, 1, 4-dichloro-, one of the main components found in the extract of *S. columbabria,* was reported being used as a deodorant.[32] Phytol, a key acyclic diterpene class of compounds, identified in the extract, was also reported to exhibit antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* by causing enormous damage to the bacterial cell membrane.[33] Pejin *et al.*[34] also reported strong antimicrobial activity of phytol by microdilution method against eight bacterial and eight fungal strains. In addition to its antimicrobial activity, phytol was also reported to exhibit no remarkable toxicity when treated with mouse skin cells.[35] Pregn-5-ene-3, 20-dione, a steroid detected in the extract, has been indicated to possesses potent antibacterial activity against 15 marine bacterial strains.[36] Batilol, found in the ethanol extract of *S. columbabria,* was reportedly used in cosmetics as a stabilizing ingredient and skin-conditioning agent,[37] while 3,5-Dihydroxy-6,7,8-trimethoxyflavone, a flavonoid, detected in higher amount (40.84%) in the extract, was previously reported to show moderate antifungal activity against *Candida albicans* with an minimum inhibitory concentration value of 312 µg/ML.[38] However, to the best of our knowledge, this is the first study to identify the bioactive constituents of *S. columbabria* by phytochemical screening and GC-MS. Therefore, it could be concluded from this study that ethanol extract of *S. columbabria* composed of various bioactive compounds which are known to exhibit various biological activities and may therefore be recommended as a plant of pharmaceutical importance.

## CONCLUSION

The study shows that ethanol leaf extracts of *S. columbabria* contained some phytoconstituents which are pharmacologically important and therefore could represent potential source of lead molecules with pharmacological activities for the development of new novel drug or a leading compound for the treatment of skin and other diseases. In addition, the presence of these compounds with biological activities justifies the traditional usage of *S. columbabria* for the treatment of skin and other diseases. Hence, further studies are needed to isolate individual bioactive compounds responsible for its therapeutic activity and the elucidation of their mechanism(s) of action.

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### Conflicts of interest

There are no conflicts of interest.

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