

ARTICLE

## Screening and Analysis of Methanolic Leaf Extract of *Psorospermum febrifugum* (SPACH)

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### ABSTRACT

Phytochemical components have been reported for various plants but very little information on *Psorospermum febrifugum* (SPACH). The presence of biocidal activity makes the spach of potential interest for the control of micro-organisms. Methanolic extract of the leaves of spach shows the various constituents (alkaloids, flavonoids, terpenoids, tannins, phenols, and steroids). Further investigation revealed phytoconstituents of methanolic leaf extract using gas chromatography-spectrophotometric techniques (GC-MS). Result of GC-MS analysis revealed the presence of eight (8) botanical pesticides with valuable biological activities. The GC-MS results revealed that eight (8) biocidal activities were present in spach namely: 1, 2, 3, 4- tetrachloronaphthalene, Permethrin-a, permethrin-b, cyfluthrin-b, cypermethrin-a, cypermethrin-c, and flumethrin-b. The result clearly shows that *Psorospermum febrifugum* hold phytocomponents species of botanical interest that could still be exploited.

### 1. Introduction

Biocidal pesticides are the naturally occurring secondary metabolites extracted from plant sources which can control and kill pests thus helping in agricultural pest management. Plants with bioactive compounds have been used to manage different crop pests with notable successes<sup>[1]</sup>. *Psorospermum febrifugum* is a flowering plant species in the genus *psorospermum*. It grows in Savannah areas and Africa, or appertain to ancestry Hypericaceae. *P.*

*febrifugum* is being employed in the treatment of various illnesses in Africa especially fever, skin problem, leprosy, poison antidote and purgative<sup>[2]</sup>. Lamoerde<sup>[3]</sup> investigated the use of *P. febrifugum* extract in the treatment of human immune deficiency virus (HIV) infections in Uganda. The use of *Psorospermum febrifugum* to treat skin rash has also been documented<sup>[4]</sup>. Similarly, the use of roots bark expressrate has been reported for the treatment of syphilis<sup>[5]</sup>. Secondary metabolites derived from plant sources have varieties of biological activities, structural arrangements

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and properties <sup>[6]</sup>.

The first report on *Psorospermum febrifugum* appeared in 1980 <sup>[7]</sup> where the xanthone: *psorospermin*, and a derivative of methyl *psorospermin chlorohydrins* were isolated from its ethanol root extract. Xanthones obtained from *P. febrifugum* are reported to be accountable for its antiviral as well as prevention of cancer activities in bone marrow and other blood - forming organs <sup>[8]</sup>. According to Tsafack <sup>[9]</sup> emodin is capable of producing good result against antitumor agent, with ability of treatment to provide a beneficial effect <sup>[10]</sup> and lung cancers <sup>[11]</sup>. The chemical that has a medicinal effect on the body in this plant is reported to have a negative side effect on DNA <sup>[12]</sup>. However, other species obtained from the plant are said to be successful for antimicrobial agents <sup>[12]</sup>. In this study we aimed to investigate chemical components from its methanolic extract of *P. febrifugum* using GC-MS techniques.

## 2. Materials and Methods

### Study Area

Benue state where the study was carried out is located on longitude and latitude (6°21' - 8°10' N and 7°44' E - 9°55' E) with total size of 30,955 km<sup>2</sup> and a population of 5,741,815 people. The state has 23 LGA. The state is bounded with Nasarawa to the north, Taraba to the east, Cross River to the south, Enugu to the south-West and Kogi to the west. The population density per square kilometer was 138 persons (NBS, 2017). Benue state consists of twenty-three (23) Local Government Areas and with three major ethnic groups namely: Tiv, Idoma and Igede. Seventy-five percent (75%) of these ethnic groups were predominantly farmers. The vegetation cover is mostly made up of giant grass (elephant grass) and tree species like: *Vetellaria paradoxa*, *Parkia biglobosa*, *Prosopis africana*, *Vitex doniana*, *Khaya senegalensis*, *Psorospermum febrifugum* etc. Along the banks of the River Benue are found hydromorphic soils, which are fertile for several crops cultivation which has earned the State a nick-name: "The Food-Basket of the Nation".

### 3. Collection and Authentication of Plant Material

The plant (*P. febrifugum*) was collected from the wild in Mobile Police Barracks area in Makurdi Local Government, Benue State, Nigeria. Taxonomic identification of the plant sample was by a taxonomist Dr. Namadi Sunusi of Botany Department, Faculty of Science, Ahmadu Bello University Zaria (ABU) with Voucher Specimen number 0936. A specimen was deposited in Botany Department, Joseph Sarwuan Tarka University Makurdi Benue state

(Former Federal University of Agriculture, Makurdi).

## 4. Preparation of Extracts

The leaves of *P. febrifugum* plant were rinsed with distilled de-ionized water in order to remove the adsorbed soil particles and contaminants such as dust, soot and aerosols. The leaves were dried in a shade for a period of seven days at ambient temperature and thereafter ground into powder using silica crucible pestle mortar. The powdered sample was kept inside 100 mL McCartney bottles until needed for further analysis.



Figure 1. *Psorospermum febrifugum*

Extraction was done using Soxhlet extraction techniques as reported by Abah and Egwari <sup>[13]</sup> with slight amendment. Two hundred grams (200 g) of powdered plant material was weighed and placed in the extraction thimble and 400 mL of methanol (ME) added. This was refluxed at a temperature of 64.7 °C (boiling point of methanol). Excess solvent was removed to dryness to give a crude extract (0.3 g).

## 5. Preliminary Phytochemical Screening

Screening of methanolic leaves extract of *P. febrifugum* was carried out based on interpretation of previous works <sup>[14-16]</sup>. The extract was tested for saponins, flavonoids, alkaloids, cardiac glycosides, terpenoids, phenols, tannins, steroids.

## 6. Column Chromatographic Separation

A column of 15 cm (length) × 1 cm (internal diameter) was packed first with glass wool and then with about 7.5 g of activated silica gel prepared in a slurry form in CH<sub>3</sub>CN. About 5 g of anhydrous Na<sub>2</sub>SO<sub>4</sub> was placed at the top of the column to absorb any water in the sample or the solvent. Prior to elution was done with 15 mL of CH<sub>3</sub>CN, without exposing the Na<sub>2</sub>SO<sub>4</sub> layer to air, to avoid evaporation of the silica gel adsorbent. The strenuous extract

was passed through the column and allowed to sink below the Na<sub>2</sub>SO<sub>4</sub> layer. Elution was done with 3 × 10 mL portions of CH<sub>3</sub>CN. The eluate was collected, dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness under a flow of analytical grade nitrogen (99.99%) for GC-MS analysis.

## 7. Construction of Calibration Curves

Original solutions of the Pyrethroids (1000 mgL<sup>-1</sup>) (Al-lethrin, Bifenthrin, Cyfluthrin, Cypermethrin, Permethrin, Tetramethrin, Telfluthrin, Phenothrin, Deltamethrin Resomethrin) pesticides were prepared and diluted serially to make non- identical concentrations between 0.03 µg/L and 0.05 µg/L of individual pesticides. Stock standard solutions were stored in amber coloured bottles at 4 °C in a refrigerator and working standard solutions were newly prepared prior to use. Original standard solutions of the pesticides passed through GC-MS under the set chromatographic conditions and average peak areas were marked against concentrations to obtain calibration curves of individual pesticides.

## 8. GC-MS Analysis

The dried eluates were rejuvenated with one (1) mL 2, 2, 4-trimethylpentane with the help of a Hamilton micro

syringe, exactly 1 µL of the extract was infused into the injection port of a gas chromatograph along with a mass spectrometer detector (GC-MS, Hewlett Packard 7890A series II). The column comprised of a DB-17 fused silica capillary column (30 m × 0.32 mm i.d. × 0.22 µm film thickness). The temperatures of the injector was 250 °C while that of detector was 330 °C (held for 5 min), respectively. The hot air chamber temperature programme started from 60 °C (1 min) and continued at the rate of 20 °C/min to 150 °C and at 5 °C/minute to 280 °C held for 4 min. The injection was done on a splitless injector at 200 °C and the purge activation time was 30 s. The conveyer gas was helium at 30 mL/min; and the splitless flow rate was 19.6 mL/min. The run time was 30 min. The various phyto-constituents were identified by comparing the elution time of standard pesticides with those in the samples, while each pesticide was quantified by comparing the response factor and ion quantity of the pesticides in samples with those in standard. Chemstation software was used to achieve all this.

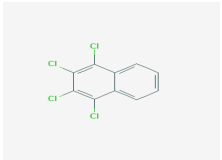
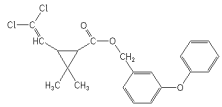
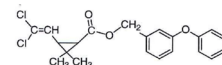
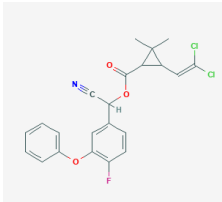
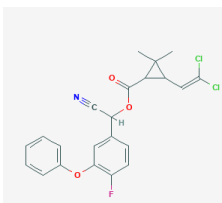
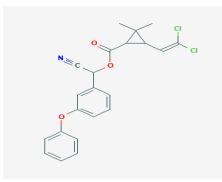
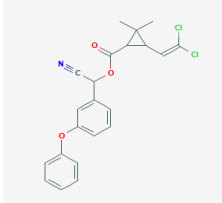
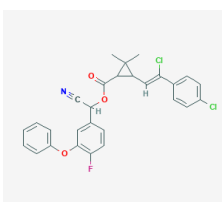
## 9. Results and Discussion

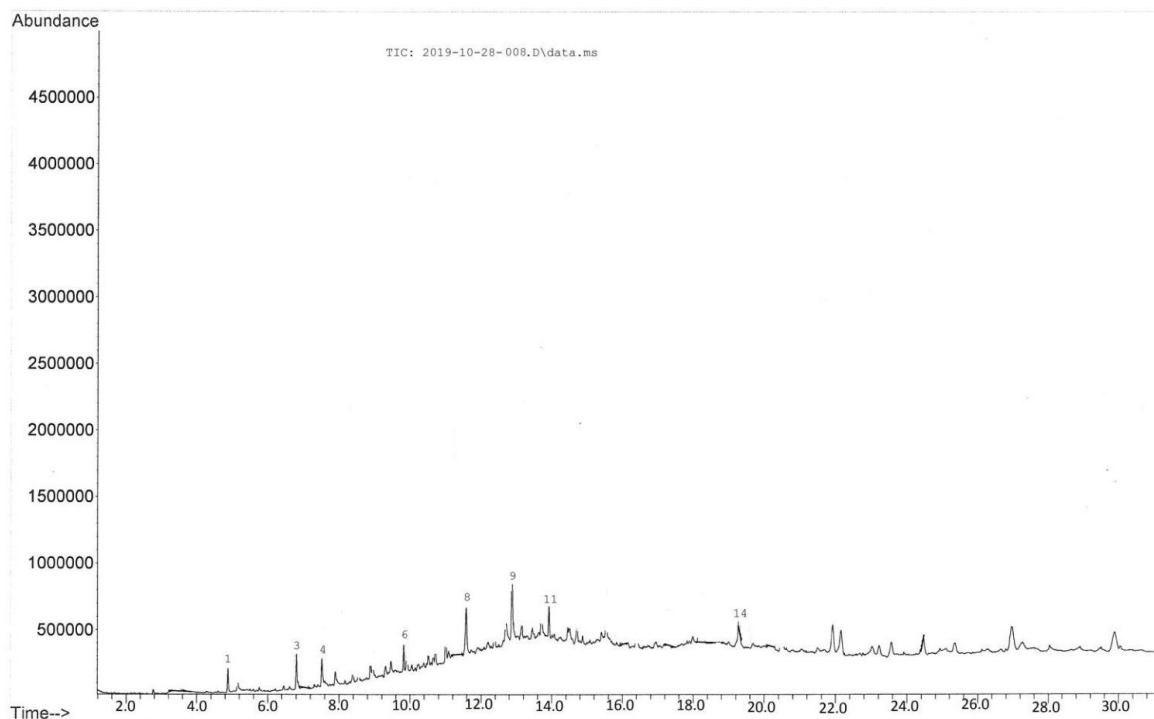
The results of the preliminary screening of *P. febrifugum* leaf methanolic extract are presented in Table 1.

**Table 1.** Preliminary Screening of *P. febrifugum* Leaf Methanolic Extract

S/no	Phytochemical	Test/Reagents	Observation	Remark
1	Saponins,	Frothing test.	The formation of an emulsion, is an indication of the presence of saponins	+
2	Flavonoids,	Dil. Ammonia and Conc H <sub>2</sub> SO <sub>4</sub> .	Yellowish coloration shows the presence of flavonoids.	+
3	Alkaloids	Draggen dorffs and Mayer's reagent..	The presence of yellowish precipitate indicate the presence of alkaloids	+
4	Cardiac Glycosides	Kedde's Keller-Kiliani	The absence of the brown ring at the interface shows the absence of deoxysugar properties of cardenolides.	-
5	Terpenoids,	Salkowski test.	The formation of reddish brown coloration at the interface shows the presence of terpenoids	+
	Phenol	Ferric chloric test	The violet or blue colouration shows the presence of phenol	+
	Tannins	Ferric chloride reagent test.	The formation of blue black colouration was observed for presence of tannins	
	Steroids	Salkowski's Liebermann- Burchard	The resultant mixture did not change colour from violet to blue or green, an indication that steroid is not present	-

**Table 2.** Phytoconstituents identified from methanolic leaf extract of *P. febrifugum* by GC-MS analysis

S/No	Name of compound	Retention time	Molecular formula	Molecular weight	Biological activity	Chemical structure
1.	1,2,3,4-Tetra Chloro-naphthalene	4.798	C <sub>10</sub> H <sub>4</sub> Cl <sub>4</sub>	265.9 g/mol	Antimicrobial/toxic	
2	Permethrin-a	6.753	C <sub>21</sub> H <sub>20</sub> Cl <sub>2</sub> O <sub>3</sub>	391.29 g/mol	Insecticide/poisonous	
3	Permethrin-b	76.753	C <sub>21</sub> H <sub>20</sub> Cl <sub>2</sub> O <sub>3</sub>	391.29 g/mol	Insecticide/poisonous	
4	Cyfluthrin-b	9.887	C <sub>22</sub> H <sub>18</sub> Cl <sub>2</sub> FN <sub>3</sub>	434.3 g/mol	Insecticide	
5	Cyfluthrin-d	11.897	C <sub>22</sub> H <sub>18</sub> Cl <sub>2</sub> FN <sub>3</sub>	434.3 g/mol	Insecticide	
6	Cypermethrin -a	13.002	C <sub>22</sub> H <sub>19</sub> Cl <sub>2</sub> N <sub>2</sub>	416.30 g/mol	Poisonous/toxic	
7	Cypermethrin-c	14.007	C <sub>22</sub> H <sub>19</sub> Cl <sub>2</sub> N <sub>2</sub>	416.30 g/mol	Poisonous/toxic	
8	Flumetrin b	19.764	C <sub>28</sub> H <sub>22</sub> Cl <sub>2</sub> F <sub>2</sub> O <sub>2</sub>	510.4 g/mol	Toxic/poisonous	



**Figure 2.** GC-MS Chromatogram of methanolic leaf extract of *P. febrifugum*

Preliminary phytochemical investigation on methanolic leaf extract of *P. febrifugum* revealed presence of species or constituents that have been reported to have antioxidant. These secondary metabolites include: alkaloids, flavonoids, tannin, phenols, saponins, terpenoids (Table 1). These are classes of plant secondary metabolites that are commonly present in plants [17-19]. A number of studies have focused on the biological activities of tannins, phenol, flavonoids and terpenoids which are antioxidants and free radical scavengers [20]. These bioactive secondary metabolites have been demonstrated to have prevented majority of cancers and diabetes, anti-inflammatory, anti-hepatotoxic, antitumor, antimicrobial and antifungal activities [21].

According to Saeed [22] Flavonoids have been implicated to be highly effective scavengers of most oxygenate molecules that are known for treatment of different diseases. Flavonoids have anti-oxidative and mucosal protective effect [23]. Vegetables with abundant flavonoids are useful on foods since they can be used to treat heart related diseases [24]. The bioavailability and, hence, constant dietary consumption of flavonoids has been documented to give pharmacologically important plasma concentrations in humans [25]. Similarly, research has reported the possible cardioprotective effects of flavonoids against ischemia reperfusion [26]. Saponins may switch on mucous membrane protective factors, while tannins lower the solubility of mucosa to chemical itching. On the other hand, sapon-

ins and tannins lower inflammation, exert astringent and protective action on the stomach mucosa, and curb excess acidity. Similarly, terpenoids and alkaloids have also been reported to have potent activity against gastric ulcers [27]. Alves-silva [28] has reported terpenoids to have effect on relax cardiovascular smooth muscle by inhibition of  $Ca^{2+}$  ions influx in vascular smooth muscle. The presence of these constituents in methanol fraction of *P. febrifugum* leaves possibly shows its numerous medicinal properties.

The result of GC-MS analysis of methanolic leaf extract of *P. febrifugum* led to identification of the following pesticides: 1,2,3,4-Tetra Chloronaphthalene, Permethrin-a, Permethrin-b, Cyfluthrin-b, Cyfluthrin-d, Cypermethrin-a, Cypermethrin-c, Flumethrin-b. Coleman [29] reported that Permethrin is a medication used in the management and treatment of scabies and pediculosis but although reports of toxicity exist when using permethrin as an insecticide, there are only a few adverse events associated with its topical use. Subramanya [30,31] reported Cyfluthrin and a synthetic cypermethrin pyrethroid insecticide which is sold as a mixture of isomers, and is highly toxic to fish, invertebrates, and insects while less toxic to humans. Flumethrin is effective against cattle ticks (*Boophilus* spp) and has been perfected as a collar combined with propoxur for the prevention of ticks and fleas in dogs [32]. Similarly, WHO [33] reported that a cohort study on workers exposed to chlorinated naphthalenes at a cable manufacturing plant found an excess of deaths from cirrhosis of the liver. From our

results, we conclude that the methanolic extract of *P. febrifugum* is a promising candidate as a botanical compound to control pest and diseases.

The results show that *Psorospermum febrifugum* contains various phytochemicals with potentials as botanical pesticides of interest. The compounds are saponins, flavonoids, alkaloids, terpenoids, phenols and tannins. Cardiac glycosides and steroids are however absent. Isolation of phytochemical constituents and subjecting them to biological activity will definitely yield fruitful results and open a new area for investigation of individual components for their botanical potency.

### Conflict of Interest

There is no conflict of interest.

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