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Phytochemical Analysis of Selected Medicinal Plants Used in Management of Anxiety and Depressive Disorders in Goma City, Democratic Republic of the Congo

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Abstract

Background: Latex of *Euphorbia abyssinica* (J. F. Gmel) (EA) and leaves of *Cleome gynandra* L.(CG), *Conyza symatrensis* (Retz) E. Walker (CS) and *Emilia coccinea* (Sims) G. Don (EC) are medicinal plant materials (MPMs) commonly used in treatment of mental illnesses (MIs) in folk medicine of Goma city, in Democratic Republic of the Congo (DRC). However, there is still limited information on phytochemical contents of these MPMs. **Objective:** To screen phytochemicals of the commonly used MPMs and to determine total contents of those with reported anxiolytic and anti-depressive activities. **Methods:** Phytochemical analysis involving both qualitative and quantitative aspects was carried out in Goma city in DRC and at Makerere University in Uganda from February to June 2022. Medicinal plant materials were extracted by methanol 90%, diethyl ether and water for eight hours replicated three times. Various qualitative tests were used in phytochemicals screening using Throth, Dragendroff, Borntrager, Salkowski, Nitric Acid, Ferric Chloride, Translucent Spot, for detecting saponins, alkaloids, carbohydrates, phytosterols, amino-acid, phenols and fatty acid respectively. Total contents of phenols, flavonoids, alkaloids and phytosteroids were determined using Folin Ciocalteau, aluminium chloride, bromocresol green-complex assay, Liberman-Burchard methods respectively. **Results:** Various phytochem-

icals were detected in investigated medicinal plants (MPs), among which terpenoids, phytosterols, steroids, phenols, amino-acids, tannins and saponins were the commonest. Among those with reported anxiolytic and anti-depressive activities, the highest concentration of alkaloids, phenols, flavonoids and phytosteroids were found in EA ether and aqueous extracts, as well as in CS aqueous extract. **Conclusion:** Findings proved that selected MPs contain potential phytochemicals with anxiolytic and anti-depressive activities.

Keywords

Phytochemicals Analysis, Medicinal Plants, Anxiety and Depressive Disorders Management

1. Introduction

Mental illnesses (MIs) rank among major health worldwide population challenges. At a global level, the proportions of world's population suffering from MIs, especially anxiety and depressive disorders are 3.6% and 4.4% respectively (World Health Organization, 2017). In African countries, 30% of the population reported with mental disorders annually, up to two third of them do not get access to adequate treatment (Sankoh et al., 2018; Group, 2007). Lack of clear mental health policy, poor health infrastructure, insufficient number of trained specialists (Psychiatrists), poor legal framework, lack of evidence-based and culturally aligned assessment and treatment as well as poverty have been identified by research findings as contributors to inadequate treatment of MIs in Africa (Monteiro, 2015).

In Democratic Republic of the Congo (DRC), in addition to the above challenges, according to several reports, sustained wars, poverty, abuse of psychoactive substances and sexual abuse, culture, poor health facilities, epidemic disease such as Ebola and sleeping sickness have exacerbated the prevalence of MIs in the country (On'okoko et al., 2010; Kangoy et al., 2016).

At cultural level, majority of the indigenous Congolese people believe that most cases of MIs have supernatural causes involving sorcery, curses and punishment from God among others (Kangoy et al., 2016). Accordingly, they believe that the illnesses are cured by casting out bad spirits in patients using charms and rituals as well as using Medicinal plants (MPs) provided by Traditional Health Practitioners (THPs) (Phani Kumar & Khanum, 2012). The current study determines whether MPs of folk medicine of Goma city contain phytochemicals with known anxiolytic and antidepressant activity that could be beneficial for the majority of patients with limited access to conventional psychiatric treatment.

The use of herbal medicine that contain various phytochemicals with different pharmacological activities on central nervous system (CNS) or brain have been reported in several studies (Phani Kumar & Khanum, 2012); herbal products

have been reported to contain mixtures of organic chemicals, including fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, terpenes etc. Polyphenols such as curcumin, ferulic acid, proanthocyanidin, quercetin, and resveratrol have demonstrated their neuroprotective effects, strongly suggesting that they can improve the symptoms of depression (Lee & Bae, 2017). Phenols, such as the fraction containing flavonoid aglycones of *Hypericum perforatum*, are among the most popular natural antidepressant used to today as they were found to provide a major part protection against lipid peroxidation (Silva et al., 2005). Flavonoids identified in *Scutellaria lateriflora* L. (American skullcap) have been traditionally used as a sedative and to treat various mental disorders like anxiety disorders (Awad et al., 2003). Berberine, an isoquinoline alkaloid of the protoberberine type found in an array of MPs has been reported to modulate neurotransmitters and their receptor systems in the brain (Kulkarni & Dhir, 2010). Preparations of *Aconitum* roots containing *Aconitum* alkaloids are employed in Chinese and Japanese medicine, and it has been reported to have an effect on the CNS. The pharmacological effects of preparations of *Aconitum* roots are attributed to several diterpenoid-alkaloids present in the plant. The main alkaloid of these plants is aconitine, a highly toxic diterpenoid-alkaloid which is known to suppress the inactivation of voltage-dependent Na^+ channels by binding to neurotoxin binding site 2 of the α-subunit of the channel protein (Ameri, 1998). β-sitosterol from MPs which has several biological activities, has been proved in various *in-vivo* and *in-vitro* studies in which it has been reported to stabilize several physiological activities like as antioxidant, CNS activity like as anti-Alzheimer, anxiolytic sedative effects and CNS depressant activity (Yadav et al., 2022).

Herbal remedies used in the management of anxiety and depression in Goma city could probably contain the above mentioned phytochemicals. However; despite their widespread use in management of MIs in Goma city, in DRC, little is known on their phytochemical contents and neuropharmacological activity. Thus, the study intended to determine whether priority MPs including *E. abyssinica*, *C. gynandra*, *C. symatrensis* and *E. coccinea* used in management of MIs in Goma city, DRC have phytochemicals with known CNS effects especially anxiolytic and antidepressant.

2. Materials and Methods

2.1. Study Design

This was laboratory experimental study. Phytochemical analysis involving both qualitative and quantitative aspects was carried out in Goma city in DRC and at Makerere University in Uganda from February to June 2022.

2.2. Selection Criteria of Medicinal Plant

Priority MPs used for the treatment of anxiety and depressive disorders in Goma city were selected on the basis of their highest use value, informant agreement ratio, and specie therapeutic potential (Kyolo et al., 2022). All MPs previously

selected as priority for anxiety and depression were included in the current study. Among these, inaccessible plant species within Goma city during the period of plants collection were excluded from the study.

2.3. Plants Collection and Identification

Cleome gynandra was obtained on purchase from Kituku market in Goma city. *E. abyssinica* latex was collected from community gardens within Goma city in DRC. However, *C. symantrensis* and *E. coccinea* were collected from bushes around Karisimbi community. All plant materials were collected from 17 February to 28 March 2022 and were identified by a taxonomist at Goma University, in DRC.

2.4. Plants Processing

The solvent chemicals for plant extraction were purchased from Kipharma, in Kigali Rwanda. Plants processing was done according to guide reported by Balamurugan et al. (2019) with some modifications according to the work. Fresh latex of *E. abyssinica* was obtained by incision of plants aerial parts. The fresh latex was dried under room temperature during four weeks and transferred to the oven set at 40 for 5 - 10 minutes to eliminate fungi and other microorganisms before extraction. Fresh aerials parts of *C. gynandra*, *C. symantrensis* and *E. coccinea* obtained from Goma city were washed under running tap water to remove durst, cleansed with distilled water and dried in the shade for seven days at room temperature (25˚C) chopped and pulverized in mortar to obtain a fine powder.

2.5. Extraction

Different solvents including diethyl ether, methanol 90% v/v and water were used. About 50 g of dried latex of *E. abyssinica* were weighed using electronic balance. The weighed dried latexes were then macerated with 200 mL of the solvents at room temperature (25˚C) for three consecutive days under magnetic agitator for eight hours a day. The macerated mixtures were filtered through a muslin cloth, filtrates were centrifuged for 20 minutes at 4000 rpm at room temperature, then filtered using filter paper number one. The extracts were stored at 4˚C if concentration was not done immediately. The resulting extracts were concentrated under reduced pressure at 40˚C - 60˚C to a syrup mass in a rotary evaporator. The syrup mass was then air-dried in a water bath and stored in a desiccator. Also, for each solvent, 100 g of dry powder of *C. gynandra*, *C. symantrensis* and *E. coccinea* were weighed. Dry powders (100 g) of each sample were mixed with 1200 mL of water. Also, 100 g of dry powders of *C. gynandra* or *E. coccinea* were mixed with 700 mL of methanol 90% v/v or diethyl ether, while 100 g of *C. symantrensis* were dissolved in 1000 mL of methanol or ether at room temperature. The mixtures were shaken for eight hours in a flask shaker using a magnetic agitator and filtered using Whatman paper number one. The resulting extracts were concentrated under reduced pressure at 40˚C - 60˚C to a mass of syrup in a rotary evaporator. The syrup mass was then air-dried in a water bath and stored in a desiccator.

2.6. Determination of Extraction Yield

The yields of evaporated dried extracts were based on dry weight that was calculated as reported by (Truong et al., 2021) from equation below:

$$Yield(\%) = \frac{W_1 \times 100}{W_2}$$

where W_1 was the weight of extract after evaporation of solvent. W_2 was the weight of the dry sample.

2.7. Phytochemical Analysis

2.7.1. Qualitative Analysis

The phytochemical analysis of the plant extracts was carried out by the standard methods provided by Evans (1966), Kala (2014); and reported by Balamurugan et al. (2019).

1) Saponins

Frothing test: A volume of 4 mL of H_2O was added to 0.2 g of extract and agitated vigorously to froth. The mixture was observed for about five minutes to determine if the froth persisted. The froth from the above reaction was taken and few drops of olive oil was added and shaken vigorously and observed for the formation of emulsion.

2) Alkaloids

Dragendroff's test: 5 mL of extract were mixed with 2 mL of hydrochloric acid. Then 1 mL of Dragendroff's reagent was added, an orange or red precipitate showed a positive result for alkaloids.

3) Glycosides

Borntrager's test: 3 mL of chloroform were added to 2 mL of filtrate and shaken. The chloroform layer was separated and 10% ammonia solution was added. The pink color indicated the presence of glycosides.

4) Phytosterols

Salkowski test: Dry extracts were dissolved in 2 mL of acetic anhydride and to which two drops of concentrated H_2SO_4 were added along the sides an array of color change that indicated the presence of phytosterols.

5) Phenols

Ferric chloride test: 10 mg of extracts were treated with few drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenols.

6) Tannins

About 0.2 g of extract was boiled in 5 mL of water in a test tube. It was then cooled under running water and filtered. A volume of 1 mL of the filtrate was added to 2 mL of water, thereafter five drops of 1% $FeCl_3$ were added and monitored for blue-black or olive-green precipitates.

7) Steroids

To 2 mL of extract, 2 mL of chloroform and 2 mL of concentrated H_2SO_4 were added, the appearance of red color and yellowish green fluorescence indicated

the presence of steroids.

8) Amino acids

Few drops of nitric acid were added to 2 mL of extract along the sides of the tube, the appearance of yellow color indicated the presence of protein and free amino acids.

9) Fatty acids

Translucent spot test: To 1 mL of the extract was mixed with 5 mL of ether. Then extracts were evaporated on a filter paper and that thereafter was dried. The appearance of transparency indicated the presence of fatty oil.

10) Terpenoids

Salkowski's test: 5 mL of each extract were mixed in 2 mL of chloroform, and 3 mL of concentrated H_2SO_4 were carefully added to form a layer. A reddish brown coloration of the inter face formed showed positive results for the presence of terpenoids.

11) Triterpenoids

10 mg of the extract were dissolved in 1 mL of chloroform; 1 mL of acetic anhydride was added following the addition of 2 mL of concentrated H_2SO_4. Formation of reddish violet color indicated the presence of triterpenoids.

2.7.2. Quantitative Analysis

Several CNS receptors have been demonstrated to interact with phytochemicals. Terpenoids, alkaloids, phenols including flavonoids have been reported with anxiolytic and antidepressant activities (Edewor-Kuponiyi, 2013). Thus, total contents of phenols, flavonoids, alkaloids and phytosteroids were quantified from plants extracts selected on basis of qualitative results.

1) Total alkaloid contents

Total content of alkaloids was measured by bromocresol green-complex assay method (Shamsa et al., 2008). From 1 mg/mL stock solution, 1 mL of test extract was gotten. To this, a phosphate buffer of 5 mL pH 4.7 and 5 mL of bromocresol green (BCG) solution were added. The mixtures were added in 4 mL of chloroform and then shaken. They were collected in a 10 mL volumetric flask and the volume was adjusted to 10 mL with chloroform. The absorbance of the bromocresol green-complex in chloroform was measured at 470 nm against blank. Atropine was used as a standard material. Total amount of alkaloids was determined using the regression equation of calibration curve and was expressed in mg of atropine equivalent to 1 g of plant extract.

Preparation of standard curve of atropine: A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 mg/mL) was prepared from a stock solution of known concentration and put in different containers. To each of these, a phosphate buffer of 5 mL pH 4.7 and 5 mL BCG solution were added. The mixture was added in 4 mL of chloroform and then shaken. Each was collected in a 10 mL volumetric flask and the volume was adjusted to 10 mL with chloroform. The absorbance of each was measured at 470 nm.

2) Total phenol contents

These were determined using Folin Ciocalteau's method (Singleton et al.,

1999) with some modifications. Briefly, to 1 mL of the sample extract we added 0.5 mL of Folin Ciocalteau reagent, followed by sodium carbonate 5% after five minutes and shaken and kept at room temperature for more five minutes. Absorbance readings were taken at 750 nm. The concentration of phenols was determined using the regression equation of calibration curve and expressed in mg of gallic acid (GAC) equivalent to 1 g of plant extract.

Preparation of standard curve of gallic acid: Different dilutions including; 25, 50, 75, 100, 125, 250, 275, 300 and 350 mg/mL were prepared from 1 mg/mL stock solution. About 1 mL from each of these solutions and a blank were taken in a test tube and 0.5 mL of Folin Ciocalteau reagent was added and shaken. 1.5 mL of 5% sodium carbonate was added after five minutes and made up to 10 mL with distilled water. Absorbance readings were measured at 750 nm.

3) Total flavonoid contents

Method reported by Brighente et al. (2007) with some slight modifications was used to measure out total flavonoid. To 0.1 mL of the stock extract, distilled water was added to make the volume to 5 mL. To this 0.3 mL of 5% $NaNO_2$ and after 5 minutes, 3 mL of 10% $AlCl_3$ were added and these were left standing for about 5 - 6 minutes. After this, 2 mL of NaOH 1 M were added and the volume was made up to 10 mL with distilled water, after which the absorbance was measured at 510 nm. Quercetin (QE) was used as a standard for construction of a calibration curve.

Preparation of standard curve of quercetin: Various concentrations of quercetin 10, 20, 40, 60, 80 and 100 mg/mL were pipetted into test tubes with the help of micropipette from 1 mg/mL solution of QE in methanol and this was used as standard. To each test tube, 0.3 mL of sodium nitrite (5% w/v) was added and after 5 minutes 0.3 mL of aluminium chloride (10% w/v) was added and left standing for five minutes. After this, 2 mL sodium hydroxide 1 M were added and volume made up to 10 mL with distilled water. The absorbance was read thereafter at 510 nm using water as blank. A standard curve of absorbance against concentration was made. Total flavonoid contents were determined using the regression equation of calibration curve and expressed in mg of QE equivalent to 1 g of plant extract.

4) Total phytosteroid contents

Liberman-Burchard's method was used to determine total phytosteroid contents (Araújo et al., 2013). To 1 mL of the extract, chloroform was added to make the volume up to 5 mL in a test tube. To this, 2 mL of Libermen-burchard's reagent was added and mixed well. The tube was covered and kept in dark for 15 minutes. The green color complex forms and the intensity were measured using a spectrophotometer at 640 nm. Cholesterol was used as a standard for constructing a calibration curve in concentrations 10, 20, 40, 60, 80 and 100 μg/mL. The standard curve obtained was used to calculate the total phytosteroid contents. Concentration of phytosteroids was expressed in mg of cholesterol equivalent to 1 g of plant extract.

2.8. Statistical Analysis

Percentage of yield, screening tests and determinations of phytochemical concentrations were replicated in three independent assays, and the results were reported as a mean ± standard deviation. Common phytochemicals contained in selected MPs were determined using frequency index. Frequency index (FI) is a relative indicator of the familiarity of a plant species that have been used in previous ethnobotanical studies (Chinsembu et al., 2015). As index, FI is a numerical scale used to compare variables with one another. It was used in current study with some modifications to found out common phytochemicals in MP species used in management of MIs in Goma city.

$$FI = \frac{FC}{N} \times 100$$

where *FC* was the number of MPs that contained a phytochemical, and *N* a total number of MPs tested. *FI* was high when there were many MPs which contained a particular phytochemical and low when were a few reports. Hence, a phytochemical was common, when its *FI* was >50% and, less common when FI was <50%.

2.9. Ethical Consideration

The study was approved by Department of Pharmacology and Therapeutics, Makerere University College of Health Sciences (MAKCHS), Biomedical Sciences Research and Ethics Biomedical Sciences Research and Ethics Committee number SIIS-841. All investigated plants were identified by a taxonomist and conserved in herbarium at Goma University, in DRC.

3. Result

3.1. Percentage Yield of Maceration Extract

Highest value of *E. abyssinica* extract yield was obtained with diethyl ether (59.92% ± 2.33%). However, highest values of *C. gynandra*, *C. symantrensis* and *E. coccinea* extract yield were obtained with water, they were 19.78% ± 3.16%, 19.91% ± 1.83%, and 19.44% ± 0.91% respectively. Lowest value of extract yields was obtained with *E. abyssinica* methanol extract (7.61% ± 0.46%), *C gynandra* diethyl ether extract (3.81% ± 0.81%), *C. symantrensis* diethyl ether extract (2.82% ± 0.18%), and *E. coccinea* diethyl ether extract (2.91% ± 0.67%) (**Table 1**).

3.2. Qualitative Analysis

Triterpenoids, phytosterols, steroids and phenols were detected in all the four MPs. Tannins were detected in *E. abyssinica* aqueous extract, *C. symantrensis* methanol extract and both methanol and aqueous extracts of *E. coccinea*. Saponins were detected in both methanol and aqueous extracts of *E. abyssinica*, and methanol extract of *C. symantrensis* and *E. coccinea*. Amino acids were detected

Table 1. Percentage yield of maceration extract.

Plant	Part used	Solvent	% yield
E. abyssinica	Latex	Diethyl ether	59.92 ± 2.33
		Methanol	7.61 ± 0.46
		Water	13.57 ± 2.07
C. gynandra	Leaf	Diethyl ether	3.81 ± 0.81
		Methanol	8.83 ± 0.81
		Water	19.78 ± 3.16
C. symatrensis	Leaf	Diethyl ether	2.82 ± 0.18
		Methanol	5.58 ± 1.43
		Water	19.91 ± 1.83
E. coccinea	Leaf	Diethyl ether	2.91 ± 0.67
		Methanol	5.37 ± 0.56
		Water	19.44 ± 0.91

n = 3.

in both aqueous and methanol extracts of *C. gynandra* and *C. symantrensis* as well as in aqueous extract of *E. abyssinica*. Fatty acids were detected in aqueous extract of *E. abyssinica* and *C. symantrensis* as well as in methanol extract of *C. symantrensis*. Alkaloids were detected in ether and methanol extract of *E. abyssinica*, and methanol extract of *E. coccinea* while glycosides were detected in ether extract of *C. symantrensis* (Table 2).

3.3. Quantitative Analysis

Results of total phytochemical contents were recorded (Figures 1-4, Table 3). The regression equation of calibration standard curve of atropine for total alkaloid contents determination was $y = 0.0024x - 0.0284$, $R^2 = 0.9833$. Standard curve of extinction against GAC concentration for total phenol contents determination was $y = 0.0013x + 0.003$, $R^2 = 0.9989$ while standard curve of extinction against QE concentration for total flavonoids contents determination was $y = 0.0096x - 0.061$, $R^2 = 0.9981$. Also, Standard curve of extinction against cholesterol concentration for total phytosteroids contents determination was $y = 0.0014x + 0.000$; $R^2 = 0.9939$. Among two plants extracts identified with alkaloids, the concentration of these phytochemicals in *E. abyssinica* ether extract was higher (79.81 ± 4.30 mg of atropine/1g of plant extract) than that of *E. coccinea* water extract (3.81 ± 0.42 mg of atropine/1g of plant extract). Concentration of phenols in selected MPs varied from 72.50 ± 15.79 to 221.82 ± 3.73 mg of GAC equivalent to 1 g of plant extract while total contents of flavonoids varied from 7.00 ± 0.42 to 27.10 ± 0.52 mg QE equivalent to 1 g of extract. The highest concentrations of phenols and flavonoids were recorded in EA water extracts.

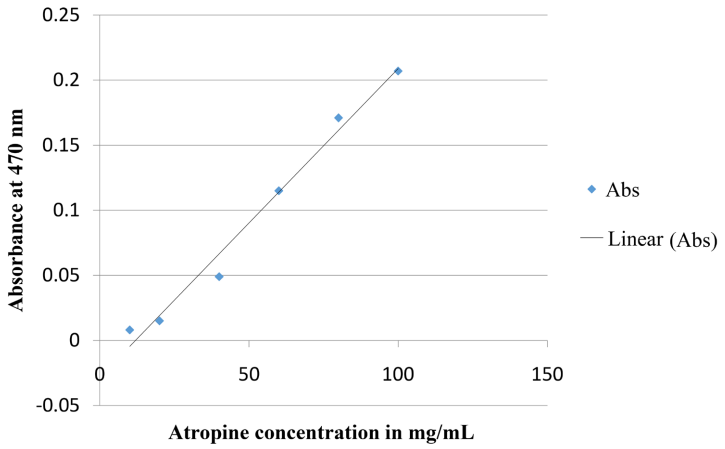

Figure 1. Standard curve of extinction against atropine concentration for total alkaloid contents determination $y = 0.0024x - 0.0284$, $R^2 = 0.9833$. Abs: Absorbance.

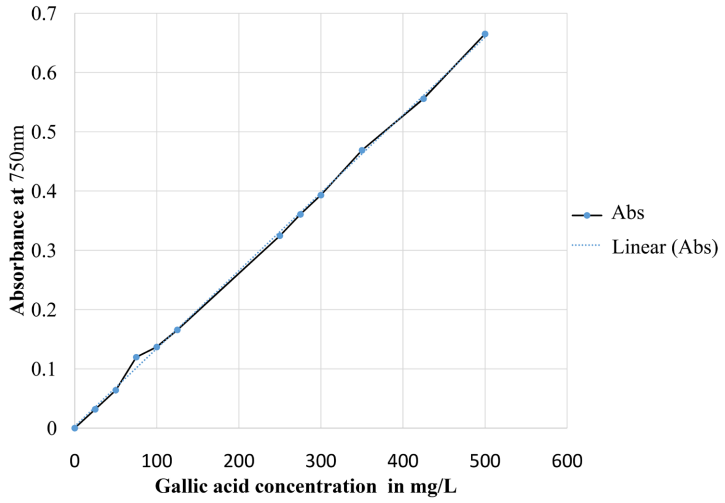

Figure 2. Standard curve of extinction against gallic acid concentration for total phenol contents determination. $y = 0.0013x + 0.003$, $R^2 = 0.9989$. Abs: Absorbance.

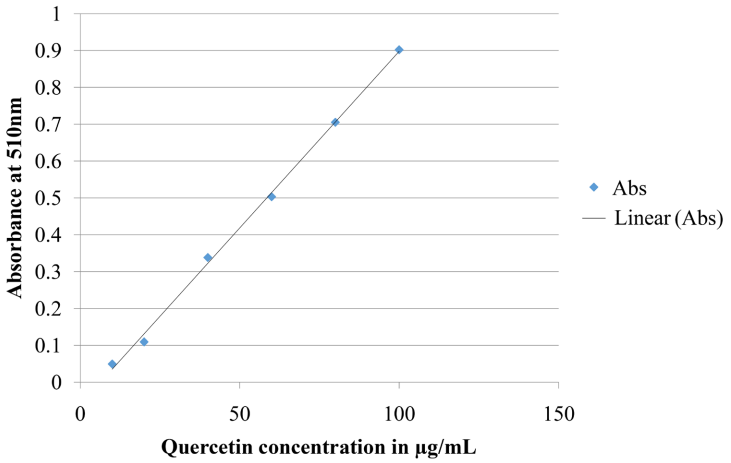

Figure 3. Standard curve of extinction against quercetin concentration for total flavonoids contents determination. $y = 0.0096x - 0.061$, $R^2 = 0.9981$. Abs: Absorbance.

Table 2. Qualitative analysis of selected plants used in management of MIs in Goma city.

Test	*E. abyssinica* latex			*C. gynandra* leaf			*C. symantrensis* leaf			*E. coccinea* leaf		
	DEE	ME	AE	DEE	ME	AE	DEE	ME	AE	DEE	ME	AE
Saponin	−	++	++	−	−	−	−	++	−	−	++	−
Alkaloid	+++	++	−	−	−	−	−	−	−	−	++	+
Glycoside	−	−	−	−	−	−	++	−	−	−	−	−
Phytosterol	++	++	++	++	+++	++	++	+++	+++	++	++	++
Phenols	−	−	+++	−	++	−	−	++	++	−	++++	++
Tannin	−	−	++	−	−	−	−	++	−	−	++	++
Steroid	++	++	++	++	−	++	+	++	−	++	++	++
Amino acid	−	−	++	+	++	++	+	++	++	−	−	−
Fatty acid	−	−	++	−	−	−	−	++	++	−	−	−
Terpenoid	++	+++	++	+++	++	++	+	++	−	++	+++	+++
Triterpenoids	+++	+++	+++	+++	++	++	+	++	++	+++	++	++

DEE: Diethyl ether extract; ME: Methanol extract; AE: Aqueous extract; +++: Strong; ++: Medium; +: Weak; −: Negative. Medium and Strong were considered as positive results in the study.

Table 3. Total contents of alkaloids, phenols, flavonoids and phytosteroids.

Plant extract	Phytochemical contents			
	Total alkaloids (ATE mg/g)	Total phenols (GAC mg/g)	Total flavonoids (QE mg/g)	Total phytosteroids (CTE mg/g)
***E. abyssinica* EE**	79.81 ± 4.30	-	-	-
***E. abyssinica* AE**	-	221.82 ± 3.73	27.10 ± 0.52	99.99 ± 5.25
***C. gynandra* ME**	-	72.50 ± 15.79	7.00 ± 0.42	208.99 ± 7.06
***C. symantrensis* AE**	-	113.87 ± 1.29	10.63 ± 0.14	228.02 ± 68.70
***E. coccinea* AE**	3.81 ± 0.42	210.45 ± 8.80	23.46 ± 0.17	212.77 ± 8.52

n = 3; EE-Ether extract; AE-Aqueous Extract; ME-methanol extract; ATE mg/g: mg of atropine equivalent to 1 g of plant extract; GAC mg/g: mg of gallic acid equivalent to 1 g of plant extract; QE mg/g: mg of quercetin equivalent to 1 g of plant extract; CTE mg/g: mg of cholesterol equivalent to 1 g of plant extract; -: not applicable.

Also, concentration of total phytosteroids varied from 99.99 ± 5.25 to 228.02 ± 68.70 mg of cholesterol equivalent to 1 g of plant extract. The highest phytosteroids contents were recorded in *C. symantrensis* aqueous extract.

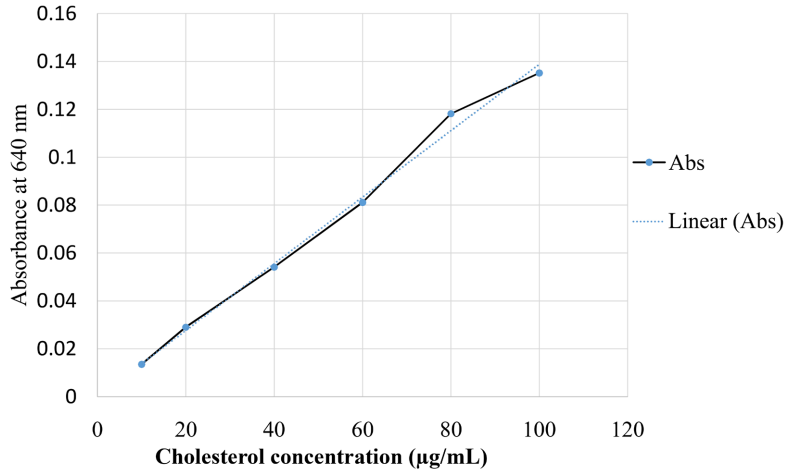

Figure 4. Standard curve of extinction against cholesterol concentration for total phytosterol contents determination. $y = 0.0014x + 0.000$; $R^2 = 0.9939$. Abs: Absorbance.

3.4. Common Phytochemicals in Selected MPs Used in Management of MIs in Goma City

Four phytochemicals including terpenoids, steroids, phenols and phytosterols were detected in all (100%) four selected MPs used in treatment of MIs in Goma city. Tannins, saponins and amino acids were detected in 75% of the MPs. However, fatty acids (50%), alkaloids (50%) and glycosides (25%) were not common to the 4 selected MPs (**Figure 5**).

4. Discussion

Triterpenoids, phytosterols, phenols, steroids were detected in all four MP extracts. Tannins were detected in *E. abyssinica* aqueous extract and *C. symantrensis* methanol extract. Saponins were detected in aqueous extract of *E. abyssinica*, and methanol extract of *C. symantrensis* and *E. coccinea*. Amino acids were detected in both aqueous and methanol extracts of *C. gynandra* and *C. symantrensis* as well as in aqueous extract of *E. Abyssinia*. Fatty acids were detected in aqueous extract of EA and CS as well as in ethanol extract of *C. symantrensis*. Alkaloids were detected in ether and methanol extract of *E. abyssinica* while glycosides were detected in ether extract of *C. symantrensis* (**Table 2**). Similar results were found by EL-Fiky et al. (2008), Saleh et al. (2018) and Tarh and Iroeggu (2019). These previous studies investigators isolated and identified β-sitosterol, alkaloids, saponins from *E. abyssinica* latex, their findings strengthen the results of current study. Also, several authors have detected phytosterols, phenols, flavonoids, steroids in *C. gynandra* ethanol extracts (Shaik et al., 2013), glycoside, tannins, flavonoids in *C. symantrensis* n-hexane and ethanol extracts (Unegbu et al., 2017) flavonoids, alkaloids, tannins, saponins, steroids, phenols and terpenoids in *E. coccinea* aqueous and ethanol extracts. These findings suggested that selected MPs used in folk medicine of Goma city for management of MIs contain interesting phytochemical compounds that could be associated with pharmacological and toxicological activities.

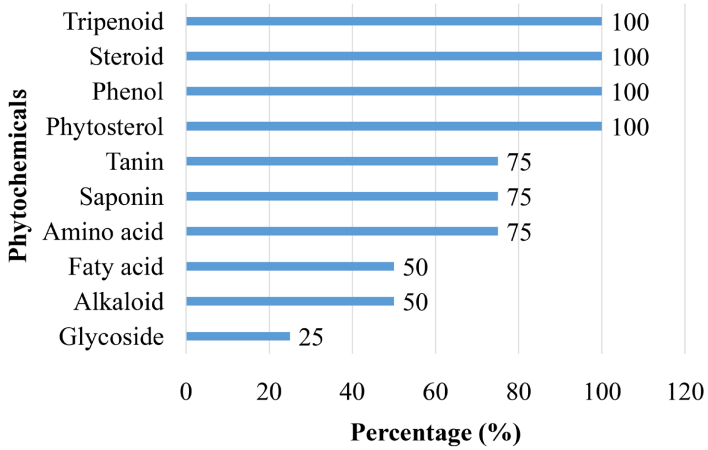

Figure 5. Frequency index (%) of selected MPs.

Highest concentration of alkaloids was found in *E. abyssinica* ether extract (**Table 3**). Similar result was found by others authors (Mengiste et al., 2014; Saleh et al., 2018), however, the previous studies were qualitative rather than quantitative. Further quantitative study is needed to certain the finding of this current study. Furthermore, it has been reported that some alkaloid substances mediate through monoaminergic and opioid receptors in CNS (Lundstrom et al., 2017). Also, study done by Edewor-Kuponiyi (2013) reported anxiolytic activity of alkaloid compounds. However little are known on pharmacological effects of *E. abyssinica* and *E. coccinea* alkaloids on CNS.

Concentration of phenols in selected MPs varied from 72.50 ± 15.79 to 221.82 ± 3.73 mg of GAC equivalent to one g of extract. Concentration of phenols including favonoids in EA water extract was higher than other investigated MPs extracts identified with that phytochemicals (**Table 3**). Phenolic compounds have been reported by numerous studies as responsible of antioxidant effects of Euphorbia species latex (Benjamaa et al., 2022). This funding was similar to study done by Saleh, Abadi and Elawad (2018). Among species of Euphorbia genus, *Euphorbia resinifera* latex water extracts has been associated with anxiolytic and antidepressant effects through GABAA receptors benzodiazepine-chloride-channel complex (Moubtakir et al., 2022), however little is known about effectiveness of *E. abyssinica* latex on MIs.

Result of current study revealed that total contents of phytosteroids varied from 99.99 ± 5.25 to 228.02 ± 68.70 mg of cholesterol equivalent to one g of investigated plant extracts. Concentrations of phytosteroids were higher in *C. symantrensis* than other selected MPs. Despite the fact that there are few studies that specifically quantified triterpenoids including phytosteroids in those MPs used in folk medicine of Goma city, in DRC, phytosteroids have been associated with neuroprotective effects in other parts of the world (Adebiyi et al., 2019).

Four phytochemicals including terpenoids, steroids, phenols and phytosterols were detected in all four selected MPs used in management of MIs in Goma city. Tannins, saponins and amino acids were detected in 75% of the MPs. Terpeno-

ids are organic chemicals derived from terpenes, which can also be termed as modified terpenes (Kurhekar, 2020). Phytosterols belong to the family of triterpenes, being constituted by a tetracyclic ring and a side chain linked to carbon 17 (Marangoni & Poli, 2010; Moreau et al., 2018). Phytosterols, also known as plant steroids or phytosteroids, are naturally occurring steroids that are found in plants with structural diversity and health-promoting uses (Moreau et al., 2018). Pronounced sedative effect and anxiolytic like-effect especially with β-sitisterol have been reported by several authors (Wagner et al., 1985; Supratman et al., 2001). Other phytochemical compounds found in current study that have been classified as subclass of terpenoids compounds (Xu & Yu, 2021) were saponins. However, there is still limited information on phytochemistry of triterpenoids isolated from the four MPs. Effects of saponins on CNS have been reported by several authors (Wu et al., 2010; Jiang et al., 2007). This finding suggested that triterpenoids present in priority MPs for management of MI in Goma city were probably phytostroids and saponins.

Flavonoids and tannins have been classified as phenolic compounds (Lattanzio, 2013) and have been reported with neuropharmacological activities such as sedative and anxiolytic effects (Jiang et al., 2007; Nisar et al., 2011). Findings suggested that phenolic compounds present in priority MPs for management of MI in Goma city were probably tannins and flavonoids. Further studies are suggested for isolation of these phytochemical components.

Some amino acids of MPs such as L-theanine (*N*-ethyl-L-glutamine) found in green tea have been suggested to possess neuroprotective and cognitive enhancing effects through increasing brain serotonin, dopamine, GABA levels and NMDA receptors (Nathan et al., 2006). This suggested that amino acids mainly present in priority MPs for management of MI in Goma city could be associated with neuropsychopharmacological effects, however there is need for further phytochemical and Pharmacological studies.

As limitation, the current study did not compare the total phytochemical compound contents between extracts of various solvents. Quantitative analysis was carried out on basis of extraction yields and qualitative analysis results. Further phytochemical studies are suggested to determine the solvent extract that contains highest phytochemical concentration.

5. Conclusion

Findings proved that selected MPs including *E. abyssinica*, *C. gynandra*, *C. symantrensis* and *E. coccinea* used in folk medicine of Goma city to treat anxiety and depression contain potential phytochemicals. Among these, terpenoids, phytosterols, steroids, phenols, amino-acids, tannins and saponins are the commonest. Regarding phytochemicals reported with anxiolytic and anti-depressive activities *E. abyssinica* ether extract and aqueous extract, and *C. gynandra* methanol contain high concentrations of alkaloids, phenols, flavonoids and phytosteroids. Further studies are suggested to identify and isolate the major compounds, and pharmacological as well as toxicological studies to determine effects on CNS and

safety of the investigated MPs respectively.

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Authors' Contributions

SKK, GSB, JO and EK designed the study. SKK, GSB and ZTK coordinated photochemistry analysis. SKK developed the photochemistry protocol analysis, undertook data analysis and wrote the manuscript. EK, GSB, JO, NM and NN contributed to the conceptual framework and reviewed the manuscript at several stages. All authors read and approved the final manuscript.

Ethics Approval/Ethics Standards

This research project was approved by research committee of the Department of Pharmacology and Therapeutics, and the School of Biomedical Sciences Research and Ethics Committee at Makerere University College of Health Sciences with approval protocol number SBS-841. The manuscript does not contain clinical studies or patient data. All investigated plants were identified by a taxonomist and conserved in herbarium at Goma University, in DRC.

Availability of Data and Material

Raw data are available as additional supporting files.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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