Comparison for the Efficacy of Column Purified Fractions of *Sinna occidentalis* and *Moringa oleifera* against *Bulinus globosus* (Intermediate Host of *Schistosoma haematobium*) from Goronyo and Shagari Dams, Sokoto State, Nigeria

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ABSTRACT

Introduction: Although, used of molluscicides to control the intermediate host of schistosomes is the best method of choice to control the spread of the snail fever among the people, synthetic molluscicides widely used are cost effective, not available and toxic to the aquatic lives and people that are completely or partially dependents on rivers or lakes water for their everyday supplement.

Aim: this research was aimed at investigation and comparison for the molluscicidal efficacy for *S. occidentalis* and *M. oleifera* leaves extracts against *B. globosus* (intermediate host of *Schistosoma haematobium*).

Methodology: Each of the plant was grinded into powder and purified through column using different solvents, *B. globosus* snails were collected from Shagari and Goronyo dams; we identified the animal using chart initially, later on the animals were confirmed as *B. globosus* by malacologist from Ahmadu Bello University Zaria, Nigeria; the Molluscicidal efficacy of the plants was tested against *B. globosus* and compared according to world health organization guidelines; mortality was calculated using Abbott's formula and analysis of variance (ANOVA) was used to determine if there is significant different between the mean mortality at $P \leq 0.05$; qualitative Phytochemical analysis was conducted to determine the chemicals presence in each plant and their combination.

Results: Present study revealed that fractions of the leaves combination showed significantly higher mortality of *B. globosus* in the present study followed by Fractions of *S. occidentalis* then that of *M. oleifera*. For the plants combination, extracts purified using methanol are highly toxic followed by fractions purified using hexane, then ethyl acetate fractions and least Molluscicidal potential was recorded in the fractions obtained using ethanol. *S. occidentalis* fractions of hexane were highly toxic followed by fractions of methanol, then fractions of ethyl acetate and least mortality was recorded from the fractions of ethanol. Similarly, for the *M. oleifera*, it was observed that, fractions of Methanol were highly toxic followed by hexane fractions then ethanol fractions and least mortality was observed in the fractions of ethyl acetate.

Conclusion: Column purified fractions for the combination for two plants leaves were highly potent for the control of *B. globosus* followed by *S. occidentalis* then *M. oleifera*.

Keywords: *Bulinus globosus*; Comparison; *Moringa oleifera*; Purified; *Sinna occidentalis*.

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I. INTRODUCTION

Schistosomiasis also called snail fever or Bilharziasis was considered among the major diseases neglected in tropical regions, caused by flukes belonging to phylum Platyhelminthes, class; trematoda and genus *Schistosoma*

[1]. Five different *Schistosoma* species were identified to cause Schistosomiasis infections [2]. Species of *Schistosoma haematobium* and *Schistosoma mansoni* occurred highly in the Africa, the South America, the Middle East and the west India whereas *Schistosoma japonicum* species are found in the Republic of China, the Indonesia and the Philippines

while *Schistosoma mekongi* and *Schistosoma intercalatum* are found in the Mekong River of South East Asia, the Middle Africa and West African parts of the world [3].

Genera of *Bulinus* and *Biomplaria* snails are known to transmit the infective stage (Cercaria) of Schistosomiasis, and infections with schistosomes are characterized by itchy, skin rash (swimmer's itch) and local swelling which can start about 24hrs after the initial infection and lasts for about 4 days [4]. Infected individuals may have fever, enlarged liver and spleen, lymph nodes and eosinophilia but this illness usually lasts for one to three weeks [5]. After months or years, infected individuals may experience painful or difficult urination (dysuria), blood in urine (hematuria), urethral obstruction, kidney damage as a result of urine obstruction (obstructive nephropathy), and enlargement of penis [6], [7].

Although schistosomiasis infection is highly increasing during rainy season [8], many human activities such as passing excreta by infected *individual* in a fresh water, construction of irrigation channels, dams and flood irrigation of crops increased infection with schistosomes in the country [9], [10].

Snail's fever affects over two hundred and forty million (240,000,000) people worldwide, many children and young adults are at greater risk in the tropics and subtropics region [11]. Over 90% of the infection occurs in sub-Saharan Africa and almost 300,000 people die annually [12], [13]. The highest prevalence of this infection is seen in Nigeria (29 million), which is closely followed by United Republic of Tanzania (19 million) then Ghana and Democratic Republic of Congo (15 million) making up the top five countries in Africa with *Schistosoma* infection [14].

Although, from Nigerian states, the most infected group is the children, everybody else is at greater risk of contracting the disease if in contact with infested water [15]. Epidemiological survey on urinary schistosomiasis in selected schools of Minna, Niger state revealed that, 12.9% children were infected [16]; 17.8% people were infected in Kano State [17]; 19.0% people in Ebonyi central of Ebonyi State were infected [18]; [19] reported that, 30.5% people were infected in Kefi town of Nasarawa State and 46.6% individuals were infected from Ogbadibo Local Government Area, Benue State [20].

Despite the fact that, In the year 2012, the Federal Ministry of Health (FMH) reported that schistosomiasis has been completely mapped out of Nigerian states (Zamfara, Kwara, Ekiti, FCT, Ondo, Gombe, Ogun, Enugu and Sokoto) [21], there are re-emergence of this diseases in some areas of these states, as preliminary study on occurrence of schistosomiasis conducted among people in Abarma Village of Gusau Local Government of Zamfara in which 74.0% were found infected [22]; moreover, Singh and [23], reported 60.8% children in some riverine areas of Sokoto were infected with schistosomiasis.

Although, among the current methods of the control of Schistosomiasis infections are reduction of morbidity and elimination through integrated measures for the control of intermediate hosts of schistosomiasis [24], synthesized chemical molluscicides used for such method were hazardous to the aquatic lives and human health [25], [26]. Due to toxic nature of synthetic molluscicides there are

needs to determine the best control methods of Schistosomiasis in order to reduce spread of the disease [27]. Moreover, in the environment like as Shagari and Goronyo where majority of the people are dependents on fresh water bodies (lakes, rivers and dam), it is highly dangerous to use synthetic molluscicides for the control of intermediate host of schistosomes.

Simma occidentalis and *Moringa oleifera* are plants been used among the people for many reasons such as food, flavor, medication among the people in different part of the world. Therefore, molluscicidal efficacy these plants against intermediate host of *S. haematobium* (*B. globosus*) were evaluated to be an increment efficient, non hazardous, efficient, cheap, and available way of controlling urinary Schistosomiasis among people of Goronyo, Shagari Local Government Areas of Sokoto State, Nigeria as well as other part of world where Schistosomiasis infections are common.

II. METHODOLOGY

A. Area of the Study

Present studies were carried out inside the General Biology laboratory, Department of Biological Sciences, Sokoto State University, Sokoto. Sokoto was in northwestern part of Nigeria; it had common borders with Republic of Niger to the North, Kebbi state of Nigeria to the south west and Zamfara state to the east. Sokoto has people population of 3,702,676, the vegetation is Sudan savannah, it lies within the longitude between 4° 8'E and 6° 5' E and latitude between 12° 0' N and 13° 54'N. the state experience four (4) to five (5) months rainfall annually (June to September or October) and 8 to 7 months dry season (from October to may), Sokoto has relative humidity vary from 10 to 25% during dry season and 51 to 75% during the rainy season with annual average temperature of 33.2 °C, the maximum daytime temperatures are generally under 40 °C most of the year, and the dryness makes the heat bearable. The warmest months are February to April, where daytime temperatures can exceed 45 °C; the highest recorded temperature is 47.2 °C. The settlement areas around Sokoto have various types of fresh water bodies such as lakes and rivers [28]. *B. globosus* snails were collected from Shagari and Goronyo dam; both Shagari and Goronyo local governments had dam namely Shagari dam and Goronyo dam, respectively. Many people around the two dams are extremely poor and dependent on fishing and other farming activities for source of their income and nutrition; they also used water from the rivers and dams for their home activities.

B. Plants Collection and Preparation of Powder

S. occidentalis and *M. oleifera* leaves used for the present study were purchased from Goronyo market, Goronyo Local government, Sokoto, the plants were mounted and maintained in herbarium press then transported to herbarium for identifications and authentications, the two plants were authenticated and identified as *S. occidentalis* and *M. oleifera* in department of biological sciences Usmanu Danfodiyo University Sokoto and voucher number was deposited as UDU/ANS/0110 and UDUS/ANS/0225 for *S. occidentalis* and *M. oleifera* respectively. Thereafter, the

fresh leaves of the plants were air dried under shade and grinded into powder using pestle and motor then stored in a sterilized container to avoid contamination [29].

C. Snail Collection

Three thousand (3000) adult snails with shall range between 11 to 13 mm were collected from Goronyo and Shagari dams of Goronyo and Shagari Local Governments respectively between 9:00am to 12:00 noon by using scoop which comprised wooden frame and net. The snails were brought to the General Biology Laboratory, Department of Biological Science, Sokoto State University in 25 liters bucket containing water from the source of collection; the length of the snails' shells were measured using a Veneer Caliper [30].

D. Preparation of Extracts

The extracts of each plant leaves were prepared using cold maceration method. Five hundred grams (500 g) of the plant was weighted and soaked in to 3000 ml of solvent in tightly covered bottles then mixed and allowed for three days (72 hour) at 20 °C, each suspension was shaken vigorously after each 24 hours. Each suspension was sieved using a mesh then filtered through a Whatmann filter paper and each of the plant filtrate was purified using column chromatography [31].

E. Column Purification

The columns were place vertically using a stand and clamp, two hundred milligram (200 mg) of cotton wool was inserted inside the column and pushed down to prevent the escape of silica gel, 140 g of silica gel with 60-120 mesh was added into the column, in each purification of each extract solvent to be used was added to flush through the column and make wetted, while flushing the column, it was slapped several times to remove the air bubbles, dropping funnel was attached on top of the column, extract to be purified was poured gently in to the column, methanol, ethanol, ethyl acetate and hexane were used differently for the purification of each of the plants extract, the mobile phase was added continuously for each plant purification until the extract eluted, fractions were collected using plastic measuring cylinder, 32 fractions were collected from each plants and each of the fraction showing similar colour with one another were matched together hence, four (4) fractions were obtained and labeled as F1, F2, F3 and F4 [32].

F. Identification and Maintenance of the Snails

Identification key was used initially to identify *B. globosus* species inside General Biology laboratory, Department of Biological Sciences, Sokoto State University Sokoto as described by [33]. Afterward, identified snails were transported to Museum of Natural History; Zoology Department of Ahmadu Bello University Zaria for confirmation of the *B. globosus* species by Malacologist, the cabinet number assigned to the identified animals was 7B, then the snails were transported back to the General Biology Laboratory, Sokoto State University for molluscicidal activity study.

G. Preparation of the Concentrations Used for the Toxicity Experiment

After preparation of stock solution, concentration of each of the plant extract was obtained using serial dilution formula ($C_1V_1=C_2V_2$), the final volume used was adjusted to obtained various concentration of the plant extract in milligram per liter. Therefore, 120 mg/l was prepared and used for the toxicity experiment of each fraction of each plant against *B. globosus*.

H. Molluscicidal Efficacy Test

Each of the plants extracts for *S. occidentalis* and *M. oleifera* (leaves) and their binary combination was tested for molluscicidal activity against *B. globosus* based on [34] guidelines procedure for molluscicidal activity test adopted by [35] with some modifications, in which ten (10) adult *B. globosus* were introduced into troughs containing 120 ppm of different fractions, the experimental animals were monitored for 96 hours inside the experimental container in starve condition, treatment in each fraction was replicated five (5) times, to confirm the mortality of the experimental animal, absence of response to the needle probe was used as an indication for the snails death. Another ten snails for each treatment was immersed in separate borehole water with the same condition without treatment to serve as control. Mortality was calculated using Abbott's formula by subtracting number of survival snails in the treated group from number of survivals from the untreated group multiplied by hundred.

I. Phytochemical Analysis

Qualitative and quantitative phytochemicals of the plant's leaves were determined using methods described by [36]-[39].

J. Statistical Analysis

Mortalities were calculated using Abbott's formula, one-way Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test were used to determine statistically significant differences between means ($P<0.05$) using IBM-SPSS software package version 20.

III. RESULTS

Molluscicidal efficacy of *S. occidentalis* and *M. oleifera* singly and their combination against *B. globosus* were time and concentration dependents, the animals were observed attempting to escape from the toxicity of the extracts immediately after introducing them into experimental container of each fraction, by distressing and becoming firmly attached to the wall of experimental container then died eventually, shells of the snails also were filled up, pores were also observed on the foot of some snails and shell's color of the snails changed from brown to dark brown and mortality was recorded after 24 hour for the period of 4 days (96 hours).

A. Molluscicidal Efficacy of *M. oleifera* against *B. globosus* after 96 Hours

Fractions of methanolic leaves extracts of *M. oleifera* showed higher mortality of *B. globosus* [where F2 showed 92.0% (9.2 ± 0.374) mortality, F3 caused 76.0 (7.6 ± 0.400)

mortality, F1 accounted for 72.0% (7.6 ± 0.200) mortality while F6 showed 56.0% (5.6 ± 0.245) mortality with significant difference ($P=0.000$) followed by fractions of hexane extract [in which F2 caused 66.0% (6.6 ± 0.245) mortality, F1 showed 56.0% (5.6 ± 0.245), F3 with 48.0% (4.8 ± 0.200) and F4 showed 40.0% (4.0 ± 0.316) with statistical significant difference ($P=0.001$)] then ethanolic extracts [whereby F4 showed 62.0% (6.2 ± 0.374) mortality, F3 showed 46.0% (4.6 ± 0.400) mortality, F2 caused 40.0% (4.0 ± 0.316) mortality while F1 showed 38.0% (3.8 ± 0.374), however, significant difference was observed ($P=0.000$)] and least mortality of *B. globosus* was recorded in the fractions of Ethyl acetate [whereby 54.0% (5.4 ± 0.400), 48.0% (4.8 ± 0.374), 40.0% (4.0 ± 0.316) and 34.0% (3.4 ± 0.400) mortalities were recorded in F1, F2, F3 and F4 respectively with statistical significant difference ($P=0.008$)] as showed in Table 1.

TABLE 1: MOLLUSCICIDAL EFFICACY OF *M. OLEIFERA* AGAINST *B. GLOBOSUS* AFTER 96 HOURS (N=10)

Fraction Number	Mean No. of Died <i>B. globosus</i>	Values of Significant at $P \leq 0.05$	Mortality of <i>B. globosus</i> (%)
Hexane Extract		0.001	
F4	4.0 ± 0.316^d		40.0
F3	4.8 ± 0.200^e		48.0
F1	5.6 ± 0.245^b		56.0
F2	6.6 ± 0.245^a		66.0
Methanol Extract		0.000	
F4	5.6 ± 0.245^c		56.0
F1	7.2 ± 0.200^b		72.0
F3	7.6 ± 0.400^b		76.0
F2	9.2 ± 0.374^a		92.0
Ethyl acetate Extract		0.008	
F4	3.4 ± 0.400^b		34.0
F3	4.0 ± 0.316^b		40.0
F2	4.8 ± 0.374^a		48.0
F1	5.4 ± 0.400^a		54.0
Ethanol Extract		0.000	
F1	3.8 ± 0.374^b		38.0
F2	4.0 ± 0.316^b		40.0
F3	4.6 ± 0.400^b		46.0
F4	6.2 ± 0.374^a		62.0
Control	0.00 ± 0.00		0.00

B. Molluscicidal Efficacy of *S. occidentalis* against *B. globosus* after 96 Hours

It was observed that, fractions of hexane extracts of *S. occidentalis* were more effective for the mortality of *B. globosus* [where 96.0% (9.60 ± 0.245), 84.0% (8.40 ± 0.245), 68.0% (6.80 ± 0.200) and 60.0% (6.00 ± 0.316) were recorded in F4, F3, F2 and F1 respectively with statistical significant difference ($P=0.000$)] followed by methanolic extract [in which 74.0% (7.40 ± 0.245), 62.0% (6.20 ± 0.374), 54.0% (5.40 ± 0.510) and 48.0% (4.80 ± 0.374) mortalities were observed in F3, F4, F1 and F2 respectively with statistical significant difference ($P=0.001$)] then fractions of ethyl acetate extracts [where F4, F3, F2, F1 showed 68.0% (6.80 ± 0.200), 60.0% (6.00 ± 0.316), 48.0% (4.80 ± 0.200) and 42.0% (4.20 ± 0.200) respectively with significant difference ($P=0.000$)], however, ethanolic extracts of *S. occidentalis* showed least mortalities of the snails [whereby 50.0% (5.00 ± 0.316), 48.0% (4.80 ± 0.200), 40.0% (4.00 ± 0.000) and

34.0% (3.40 ± 0.245) mortalities were observed in F4, F3, F2 and F1 respectively with significant difference ($P=0.000$)] (Table 2).

TABLE 2: MOLLUSCICIDAL EFFICACY OF *S. OCCIDENTALIS* AGAINST *B. GLOBOSUS* AFTER 96 HOURS (N=10)

Fraction Number	Mean No. of Died <i>B. globosus</i>	Values of Significant at $P \leq 0.05$	Mortality of <i>B. globosus</i> (%)
Ethanol extract		0.000	
F1	4.20 ± 0.200^e		42.0
F2	4.80 ± 0.200^e		48.0
F3	6.00 ± 0.316^b		60.0
F4	6.80 ± 0.200^a		68.0
Hexane Extract		0.000	
F1	6.00 ± 0.316^d		60.0
F2	6.80 ± 0.200^e		68.0
F3	8.40 ± 0.245^b		87.0
F4	9.60 ± 0.245^a		96.0
Methanol Extract		0.001	
F1	4.80 ± 0.374^d		48.0
F2	5.40 ± 0.510^e		54.0
F3	6.20 ± 0.374^b		62.0
F4	7.40 ± 0.245^a		74.0
Ethyl acetate extract		0.000	
F1	3.40 ± 0.245^b		34.0
F2	4.00 ± 0.000^b		40.0
F3	4.80 ± 0.200^a		48.0
F4	5.00 ± 0.316^a		50.0
Control	0.00 ± 0.000		0.0

C. Molluscicidal Efficacy for the Combination of the Two Plants against *B. globosus* after 96 Hours

After 96 hours in to various fractions of different solvents of the plants combination, it was reported that, fractions of Hexane extracts showed highest mortality of the snail [where F2 and F3 showed 100% (10.00 ± 0.000) mortality of the snails while F1 and F4 showed 98.0% (9.80 ± 0.200) and 82.0% (8.20 ± 0.200) mortalities respectively with statistical significant difference ($P=0.000$)] followed by fractions of methanol with 100% (10.00 ± 0.000), 90.0% (9.00 ± 0.316), 76.0% (7.60 ± 0.510) and 66.0% (6.60 ± 0.245) mortalities in F4, F3, F2 and F1 respectively with significant difference ($P=0.000$); then fractions of ethyl acetate [which showed mortality of 98.0% (9.80 ± 0.200), 78.0% (7.80 ± 0.200), 72.0% (7.20 ± 0.200) and 64.0% (6.40 ± 0.245) for F4, F3, F2 and F1 respectively with significant difference ($P=0.000$), while least mortality was reported in ethanol [where F4, F3, F2 and F1 showed 96.0% (9.60 ± 0.245), 84.0% (8.40 ± 0.510), 76.0% (7.60 ± 0.245) and 64.0% (6.40 ± 0.245) mortality was reported respectively with significant difference ($P=0.000$)] as reported in Table 3.

D. Phytochemical Constituents of the Plants

Phytochemical analysis of *S. occidentalis* revealed that, alkaloids, flavonoids, saponins, tannins, and glycosides were present in excess, cardiac glycosides and volatile oils were moderately present while balsams, steroids and antraquinones quietly present whereas analysis phytochemical constituents of *M. oleifera* showed excess flavonoids, saponins, glycosides and antraquinones. Although, alkaloids, volatile oils, tannins and steroids were moderately presents, balsams and cardiac glycosides were presents in least quantities while all the phytochemicals were found in excess in the combination of the two plants

with exception of volatile oils and balsams which were found moderately present as shown in Table 4.

TABLE 3: MOLLUSCICIDAL EFFICACY FOR THE COMBINATION OF TWO PLANTS AGAINST *B. GLOBOSUS* AFTER 96 HOURS (N=10)

Fraction Number	Mean No. of Died <i>B. globosus</i>	Values of Significant at $P \leq 0.05$	Mortality of <i>B. globosus</i> (%)
Ethyl acetate Extract		0.000	
F1	6.40±0.245 ^c		64.0
F2	7.20±0.200 ^b		72.0
F3	7.80±0.200 ^b		78.0
F4	9.80±0.200 ^a		98.0
Hexane Extract		0.000	
F1	8.20±0.200 ^b		82.0
F2	9.80±0.200 ^a		98.0
F3	10.00±0.000 ^a		100.0
F4	10.00±0.000 ^a		100.0
Methanol Extract		0.000	
F1	6.60±0.245 ^d		66.0
F2	7.60±0.510 ^c		76.0
F3	9.00±0.316 ^b		90.0
F4	10.00±0.000 ^a		100.0
Ethanol extract		0.000	
F1	6.40±0.245 ^c		64.0
F2	7.60±0.245 ^b		76.0
F3	8.40±0.510 ^b		84.0
F4	9.60±0.245 ^a		96.0
Control	0.00±0.000		0.0

NOTE: From each of the above tables, mean numbers of the snail died were expressed as Mean±SEM of five replicates. Value in row with different superscript differs significantly at $p \leq 0.05$ level using One Way ANOVA followed by Duncan Multiple Range Test. N=Number of *Bulinus globosus* inside each treatment which is ten (10).

TABLE 4: PHYTOCHEMICAL ANALYSIS OF *S. OCCIDENTALIS* AND *M. OLEIFERA*

Phytochemical Components	<i>S. occidentalis</i>	<i>M. oleifera</i>	Combination of the plants
Alkaloids	+++	++	+++
Flavonoids	+++	+++	+++
Saponins	+++	+++	+++
Tannins	+++	++	+++
Glycosides	+++	+++	+++
Cardiac glycosides	++	+	+++
Volatile oils	++	+	++
Balsams	+	+	++
Steroids	+	++	+++
Antraquinones	+	++	+++

Key:

Excess Present = +++

Moderate Present = ++

Quite Present = +

IV. DISCUSSION

Molluscicidal effect of *S. occidentalis* and *M. oleifera* leaves extracts singly and their combination against experimental animals were time dependents, because the higher the increase in time the higher the rate of mortality observed in the treated groups, the present finding was not contrary to the report of [40] who observed that molluscicidal toxicity of *Agave filifera* extract on *B. alexandrina* was time dependents, similarly [41], observed that, efficacy of *Bauhinia variegata* and *Mimusop selengi* extracts against *Lymnaea acuminata* was time and concentration dependents, nevertheless, [42], observed that,

molluscicidal potential of *Ammivis naga* and *Chenopodium ambrosioides* against *B. alenxendrana* were attributed due to increased time of exposure and concentration dependents. This was also supported by [43] and [44]. [45], also stated that, molluscicidal potency of *M. oleifera* leaf extracts and *Momordica charantia* fruits against snail *Lymnaea acuminata* were time and concentration dependents.

Experimental animals' distresses, changing colour and filling up of the shells as well as swelled pores on the foot of the snail were due to toxicity of the plants extracts on *B. globosus*. According to [46], the inference from these observations was that, the tissue of the cephalopodal mass had accumulated water, which caused haemorrhage at lethal concentration of the active plant extracts that caused snails' distress, filling up of the shell and pores on the foot of the snail. Nevertheless, the observation made in this study of the foot-sole surface epithelium by preventing its normal osmoregulatory function [47]. This finding was similar with the reports of [48], similarly, [48], observed movement of snails to the side of the container in an attempt to escape from the test media containing *Althernan therasessils* treated water. [49], also reported that, the snails showed several behavioral responses, including the "distress syndrome" described for other Planorbid species, indicative of intoxication and they stated that, swelling of the tissues was not restricted to the tentacles, but involved the whole cephalopodal mass.

Although, significant difference was observed between mean mortality of the snails treated in fractions of each plant used after 96 hours of the experimental set up, higher mortalities were recorded in combination of the plants extracts and when compared to the single forms of the two plants, the combination of the two plants had higher toxicity against *B. globosus* than the single form of the plants. Although this report is contrary to [50], who observed that, use of plant extracts singly, especially in the case of *Sinna occidentalis* is more effective than combination of two or more plants against the test organism (*Lymnaea ovum*) snail. It was similar to observation of [51], who reported at, the use of plant in combination, is more effective than individual plants towards snail control. Similarly, this finding was also in agreement with the work of [52] who reported that, combination of two plants showed more effectiveness in the control of *Biomphalaria alexandrina*. In addition, it suggested that combinations of equal parts of custard apple seed powder with oil from *Cedarcedrus*, *Deodara Roxh*, and *Azctdirachta indica*, was more toxic than the individual components of these plants [53]. It was observed that, powdered seed of *Annona Squamosa* and *Lawsonia inermis* were potent against *Lymnaea acuminata* and *Indoplanorbi sexustus* and combination of these plants increased higher molluscicidal potential against the animals than it single forms [54].

Phytochemical observed into tested plants could be consider as one of the major factor that caused higher mortality of the experimental animals because animals treated with the plants combination showed higher mortality while potent phytochemicals like alkaloids, flavoides, saponins, glycosides and tannins were recovered in higher amount in the combination. This was in line with the report of some researchers among which included, [55] who reported that, potency of the plants against snails were due

to higher amount of saponins compounds, similarly, [56], observed that, higher amount of tannins, flavonoides glycosides and alkaloids caused higher molluscicidal activity against snail species.

V. CONCLUSION AND RECOMMENDATIONS

Fractions extracted from the combination of the plants leaves had higher molluscicidal potential against *B. globosus* snails followed by *S. occidentalis* and least molluscicidal efficacy was observed in the column purified fractions of *M. oleifera*. Phytochemicals analysis showed that, compounds that are responsible for the higher mortality of the snails were recovered in excess amount in combination of the plants followed by *S. occidentalis* then *M. oleifera*. Hence it was recommended that, the combination of these plants should be used in control of *B. globosus* and further study should be carryout to investigate the mode of action of the plants against *B. globosus* by conducting histological and biochemical examinations on some body parts of the experimental animals.

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