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Preliminary Study of the Phytochemical Constituents of *Cassytha filiformis* (Love Vine)

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Abstract: In this research work, cold extraction was used to extract the phytochemical constituents of *Cassytta filiformis* using methanol and petroleum ether (pet. ether) as solvent. Screening tests carried out on the raw sample and the crude extract using standard procedures indicated the presence of alkaloids, glycosides, flavonoids, saponins and tannins. Thin layer chromatographic result showed that the methanol and petroleum ether extracts contain at least four and two substances (compounds) respectively. It also revealed that the best eluting solvents system for the plant constituents is the mixture of n-butanol, acetic acid and water (in the ratio 5:2:3).

Key words: Phytochemical · *Cassytha filiformis* · Methanol and Petroleum ether

INTRODUCTION

For ages, man has been searching for substances that would relief pains and cure him and his family of different illnesses. This he does by exploring the nature especially plants and animal. Many curative agents have been discovered via trial and error. More than seventy-five percent of synthetic drugs in use today are modifications and improvements of chemically active constituents of plants, which are isolated, characterized and then synthesized [1, 2, 3]. The medicinally active substance found in plants in general exists in the form of both primary and secondary metabolites such as proteins, fats, glycosides, amino acids, alkaloids, flavonoids, carbohydrates, tannins and saponins [4, 5]. Plants such as such as *Cassytha filiforms,* which is a perennial, parasitic, herbaceous and leafless plant, are known to have pharmacological activities [6, 13]. It is a medicinal plant traditionally used for the treatment of cancer, African trypanosomiasis and many other related diseases. This because *Cassitta filiformis* has a number of biologically active chemical compounds with potential human health applications. *Cassitta filiformis* has a high aporphine alkaloids as well as some other chemically active substance that plays an important role in pharmacology [7, 8 and 15]. For instance, from *Cassitta filiformis*, ocoteine is extracted which is an alpha – adrenoceptor that has the ability to block thoracic aorta in rats. This chemistry of *Cassitta filiformis* has potential application for inhibiting certain carcinomas such as prostate cancer. It has been reported in Taiwan of application of *Cassitta filiformis,* intreatment of gonorrhea and kidney ailment [9, 10, 11, 20]. It has also been reported in India of the use of dried *Cassitta filiformis* to cure intestinal ailments. *Cassitta filiformis* is known to help in relieving pains and arthritis condition as diuretics [12, 29]. Modern midwives recommend taking the juice made from crushed vines for 4 weeks before the expected date of birth in order to ease labour pains and to quicken labour times and lubricate the birth canal [13, 20]. In summary, the study suggested that aqueous extract of *Cassytha filiformis* administered at normal therapeutic doses is not likely to produce severe toxic effect on some organs or haematological and biochemical indices in rat. In summary, the chemical constituents of aqueous extract of *Cassytha filiformis* have anti-cancer and anti-trypanosome in addition to anti-platelet aggregation activity. It is also known to have alpha – 1 adrenoceptor blocking agent acivity as well as vasorelaxing effects and diuretic activity [16, 17, 18, 26].

It is identified by different names such as love vine in English, *Schlingfaden* in German and *bejucodorado* in Spanish [1 and 21]. However in Nigeria it is known *Egbu mbefu* among Ezillo people where this present work was done. The aim of this work is therefore to analyze *Cassitta filiformis* for its phytochemical constituents.

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MATERIALS AND METHODS

Sample Collection: The stems of *Cassytha filiforms* were collected from Obichiri village in Ezillo of Ishielu L.G.A, Ebonyi State and the plant was authenticated in the Department of Applied Biology, Ebonyi State University and Abakaliki.

Sample Preparation: About 160g of the fresh sample was washed with distilled water to remove sand and other environmental contaminants. It was afterwards partly air dried and sun dried for 3 weeks and ground to powder. The ground sample was weighed and stored in pretreated polythene bag until needed for analysis in accordance to the method described by….. [1] and…[21].

Solvent Extraction: This was done using petroleum spirit (pet. ether) and methanol as the solvents as describe by many researchers [1, 27, 28]. The first extraction was carried out on 40g of the powdered sample with 250ml of petroleum spirit (pet. ether). The powdered sample was dissolved / soaked in the solvent (pet. ether) and the solution was shaken (hand shaking) for about 10 minutes afterwards the solution was allowed to stand for 48 hours (2days). The mixture was filtered and further extraction was carried on the residue using 100ml of the same solvent (pet. ether) this later mixture was allowed to stand for 24 hours and then filtered. The petroleum ether extracts (both the first and second filtrate) was stored in a reagent bottle and labeled fraction. The second extraction was carried out with 25oml methanol on the same amount (40g) of the powdered sample and same process as in fraction I was repeated and the extract (methanol extract) was stored in another reagent bottle and labelled fraction

Preparation of the Reagents Used Mayer's Reagents: Mayer's Reagent is used in qualitative test for alkaloids, which may be present in a plant extract. It is a colourless solution which reacts with alkaloids to give a white precipitate. Mayer's agent consists of $HgCl_2$ 1.36g, KI 5.00g, Distilled water 100mls. These quantities were however, scaled up by a valve of 2.5 units for the purpose of this practical. Hence 3.40g of $HgCl_2$, 12.50g of KI and 250ml distilled water was used to prepare the Mayer's reagent.

Dragnetdroff's Reagent: Dragendroff's reagent is used in the qualitative test for alkaloids, which may be present in a plant extract. It is a violet coloured solute which contains some precipitate.

The precipitate is allowed to settle or filtered off, so as not interfere in the test. The reaction between the dranendroff's agent and an alkaloid give an orange precipitate.

Dragendroff's reagent consist of; $BiCl_3$ 500g; KI 5.00g; Distilled water 500mls.

These quantities were scaled down to reduce the volume of distilled water, since 500mls were considered too much for these spot test. The volume of distilled water was scaled down by a value of 2.0 units. Hence, 2.50g $BiCl_3$ 2.50g KI and 250ml distilled water gave the dragendroff's reagent.

Fehling's Solution: Fehling's is a mixture of equal volume of Fehling's solution and Fehling's solution B. Fehling's solution is used in the test or simple sugar E.g. glucose. Fehling solution undergoes a colour re action with simple sugar to give a brick-red precipitate.

Solution A: 69.28g of $CuSO_4.5H_2O$ was weighed out dissolved in 50mls distilled water. The solution was made up to 1 litre.

Solution B: 346.00g of $NaKC_4H4O_6.4H_20$ and 120.00g NaOH pellets were weighted and dissolved in 50mls of distilled water. The resulting solution was made up of 1 litre.

Ferric Chloride ($FeCl_3$): Ferric chloride is used in the test of varied organic compounds, e.g. in the test for phenols and tannins. The use of $FeCl_3$ in this work is for the test for tannins. $FeCl_3$ undergoes a colour reaction with tannins, to give a greenish-black colouration. Ferric chloride solution sometimes contains a large excess of HCl which could interfere the reactions. It was therefore neutralized with dilute NaOH solution, drop by drop, until a small but permanent precipitate of ferric hydroxide was obtained. The precipitate was filtered off through a small fluted filter paper and the clear filtrate was used in the tests.

Molisch's Reagents: Molisch's reagent is used for the test of the presence of carbohydrates. It gives a purple colouration at the interface between the Molisch's reagent layer and the carbohydrates layer. Molisch reagent is a 1% alcoholic solution of α-naphthol. For the alcohol, ethanol was used.

Hagner's Reagent: Hagner's reagent is also called picric acid reagent. It is used for the detection of alkaloids. Hagner's reagent reacts with an alkaloid to give a yellowish precipitate. Hanger's reagent is a saturated solution in 2mis of distilled water.

Wagner's Reagents: Wagner's reagent is used for the test of alkaloids which may be present in a plant extract. The addition of Wenger's agents to an alkaloid results into a brown precipitate. Wagner's reagent is a solution of Iodine in potassium iodine. 3 ml of iodine was missed with 3g of KI to obtain the solution.

Thin Layer Chromatography Analysis: In the TLC analysis, the stationary phase is a solid (Silica gel) and the mobile phase is a liquid. The liquid is made up of a mixture of different solvents. Four solvents systems with varying ratios were tried in order to ascertain which one gives the best separation. The four solvent systems tried (with their respective varying ratios given in ml) were:

1	n-Butanol:	Acetic acid:	water in the ratios	(a) 5:1:4	(a) 4:2:4
					(a) 5:2:3
2	Chloroform:	Methanol:	water in the ratios	(a) 7:2:1	
					(a) 6:2:2
					(a) 6:3:1
3	n-Butanol:	Ethyl acetate:	water in the ratios	(a) 5:1:1	
					(a) 5:2:3
					(a) 5:1:4
4	n-Butanol:	water in the ratios		(a) 5:4	
					(a) 6:3

The TLC Procedure [30]: A spot of each of the extract to be separated was placed near one edge of the TLC plate with a micro-pipette. After these solvents had evaporated from these spots, the TLC plate was inserted into a chromatographic tank containing the elution solvent (e.g. mixture of n-Butanol, ethyl acetate and water in varying ratios) and the container was tightly covered. The solvents ascended the plate by capillary migration (or action). When the solvent level had risen up in the plate, the plate was removed from the container and allowed to dry. The position of the spots on the plate was determined and the Rf values of the various spots was calculated.

The intensity of a spot is a measure of the amount of material present in the sample. In this work, all the extracts, when concentrated were dark brown in colour.

This research work used Silica gel as the stationary phase. The stationary phase (Silica gel) can be considered to be polar. In all the cases, mixture of solvents in different (varying) ratios was used. Four solvent systems in varying ratios were tried in order to ascertain the one that gives the best separation (i.e highest number of well separated spots). The four solvent systems as well as the results they produced were:

(a)n-butanol: Acetic acid:H_2O: The first solvent system used was n-butanol, Acetic acid and water and three different ratios were tried.

(i)Ratio 5: 1: 4: The first ratio was 5:1:4 and this gave 3 spots with Rf values as 0.21, 0.55 and 0.85 respectively for methanol extract and gave 2 spot with Rf values of 0.87 and 0.93 for petroleum ether extract. This 'high' Rf values of the spots from Pet. ether extract indicates that petroleum ether (Pet. ether), being a dipolar aprotic solvent (i.e weakly polar solvent) was only able to extract the least polar compounds in the sample thereby giving rise to spots with high Rf values. Also considering the solvent system used, acetic acid and water which constitutes 50% of the solvent system (mobile phase) is more polar than the n-butanol constituting the remaining 50%, hence this gave 3 spots for methanol extract and 2 spots for Pet. Ether extract.

(ii) Ratio 4: 2: 4: Second analysis was carried out using the same solvents but with the ratio 4: 2: 4. This mixture gave 60% very polar (i.e from water and acetic acid) and 40% slightly polar from (n-butanol) solvents system. This solvent system however gave 3 spots for methanol extract with Rf values of 0.45, 0.71, 0.91 and gave 1 spot for petroleum ether extract with Rf value of 0.90. The resulting 'one' spot from the Pet. ether extract may be due to the high polar nature of the solvent system that could not separate the non-polar compounds in the Pet. ether extract thereby giving rise to a single spot with high Rf values.

(i)Ratio 5: 2: 3: The third ratio tried from the solvents, n-butanol, Acetic acid and water was 5: 2: 3. This gave a mixture that is partly polar and partly non-polar. This mixture was able to produce 4 well separated spots for methanol extract with the Rf values of 0.33, 0.60, 0.74 and 0.88. The resulting higher number of spots from this ratio of solvents (when compared with the previous ratios) may be due to the fact that the mixture provided a polarity environment that was able to solvate and separate the various components in the sample into four different spots. The mixture also gave 2 spots with high Rf values of 0.93 and 0.95 for Petroleum ether extract.

(b)Chloroform: Methanol Water: The second solvent system used was a mixture of chloroform, methanol and water. Among these solvents, chloroform is less polar

than methanol and water. The solvent ratios tried were (i) Ratio 7:2:1 (ii) Ratio 6:2:2 (iii) Ratio 6:3:1. These solvent ratio gave a mixture that is weakly polar as they contain more percentage of chloroform (which is weakly polar) than methanol and water (which are very polar). As a result, TLC analysis done with these solvent mixtures gave 2 spot (in all the three cases) for methanol extract with high Rf values ranging from 0.60 to 0.96. it also gave 1 spot for petroleum ether extract (in all the three cases) ranging from 0.95 to 0.96. These spots with high Rf value resulted from the use of weakly polar solvent systems which was only able to separate the weakly polar compounds thereby giving rise to spots with high Rf values.

(c) n-Butanol: Ethyl acetate: water: For this third solvent system, three ratios were tried as well. These ratios are: (1)Ratio 5:1:1 (2) Ratio 5:2:3 and (3) Ratio 5:1:4. Among the three solvents, water have the highest polarity than ethyl acetate and n-butanol, hence the first and second ratio gave a mixture that is weakly polar (since the percentage of water is low). As a result, solvent mixture formed from the first and second ratio gave 2 spot for methanol extract with a high Rf value ranging from 0.61 to 0.93 in both cases. It also gave 1 spot for pet ether extract (in both cases) with an Rf value ranging from 0.85 to 0.91. These high Rf values shows that the solvent mixture was only able to solvate the weakly polar compounds. On the other hand the third solvent ratio gave a mixture that is more polar than the former due to the increase in the volume of water. As a result, 2 sports each was formed from the methanol and petroleum extract.

(d). n- Butanol: Water: In this fourth solvent system, water is more polar than n-Butanol. The first solvent ratio used was the Ratio 5:4. This gave 2 spot for methanol extract with the Rf values of 0.88. The second ratio tried for this solvents system was the Ratio 6:3. The increase in the volume of n-butanol from 5ml to 6ml and the reduction of the volume of water from4ml to 3mls made the mixture to be less polar than the previous ratio. As a result, only one spot was formed from each of the methanol and petroleum ether extract.

RESULTS AND DISCUSSIONS

Table 1: Preliminary Qualitative Photochemical Screening of the Crude Sample of *Cassytha filiformis*

S/N	TEST	OBSERVATION	INFERENCE
	FOR ALKALOIDS		
	0.5g of sample was macerated using 20mls ethanol. The extract was filtered off the plant fibers. The filtrate was divided into 4 portions.		
I	Portion 1 of the extract was acidified with 2M HCl + 3 drops of Mayer's reagent.	White precipitate	Alkaloids presence
II	2nd portion of the extract was added few drops Dragendroff's reagent	Orange colouration with precipitate.	Alkaloids present
III	3rd portion of the extract was added few drops of Wagner's reagent.	Reddish brown precipitate	Alkaloids presence
IV	4th portion of the extract was added few drops Hagner's reagent.	Yellow colouration	Alkaloids confirmed presence
	FOR SAPONIN		
(a)	0.5g sample + 20mls distilled water. Boiled for 2mins. Filtered hot and cooled. Filtrate was vigorously shaken.	Persistent frothing. It lasted for long time	Saponins present
(b)	A portion of the filtrate was added to Arachis oil	Heavy emulsions. The emulsion lasted for long time	Saponins confirmed present
	FOR FLAVONOIDS		
(a)	Filtrate from saponin test was added to 3 drops of 1% $AlCl_3$ solution.	Light yellow colouration	Flavonoids present.
(b)	0.3g of the sample was added 10mls of ethyl acetate and boiled. It was filtered and cooled. To 3mls of the filtrate were added 2mls of NH_3 solution	Pale yellow colouration in the aqueous layer	Flavonoids present
	FOR TANNINS		
A	Few drops of $FeCl_3$ were added to 5mls of the filtrate from saponin test	A greenish-black Colouration Appeared red	Tannins present
B	3mls of filtrate from saponing test were added 3 drops of lead II acetate	Yellow-brown gelatinous precipitate	Tannins confirmed present
	FOR GLYCOSIDES		
	0.3g of sample was added 5mls of dill.H_2S0_4 in a test tube. The test tube was placed in boiling water for 5mins. The solution was cooled and neutralized with 20% Na0H. To 5mls of the solution were added 10mls of Fehlings solution	A brick-red precipitate	Glycosides present
	Sodium picrate paper was dipped into the solution	The sodium picrate paper turned redish brown	Glycosides confirmed

Table 2: The Preliminary Phytochemical Analysis of the Crude Sample

Phytochemicals	Inference
Alkaloid	+ +
Saponin	+ +
Flavonoid	+
Tannins	+
Glycoside	+ +

+ + = High concentration, + = Low concentration, - = absence

Table 3: Result of the Phytochemical Analysis of the Methanol and Petroleum ether extract

	INFERENCE	
PHYTOCHEMICALS	Methanol extract	Petroleum ether extract
Alkaloid	+ +	—
Saponin	+ +	+
Flavonoid	+	++
Tannins	+	+
Glycoside	+ +	+ +

Table 4: TLC Chromatography Results

S/N	Solvent system	Solvent ratio	Sample extract	No of spots	Colours of spots 1 and 2	R_f values of Spots 1 and 2
1	n-Butanol (n-BUOH)	5	1Methanol Extract	3	1A: Pale Brown	1A = 0.21cm
	Acetic acid	1	2Pet. Ether extract	2	1B: Orange	1B = 0.55cm
	Water (H_2O)	4			1C: Pale yellow	1C = 0.85cm
					2A: Pale yellow	2A = 0.87cm
					2B: Greenish yellow	2B = 0.93cm
2	n-Butanol (n-BUOH)	4	1Methanol Extract	2	1A: Brown	1A = 0.45cm
	Acetic acid	2	2Pet. Ether extract	1	1B: Orange	1B = 0.71cm
	Water (H_2O)	4			1C: Greenish yellow	1C = 0.91cm
					2A: Greenish Yellow	2A = 0.90cm
3	n-Butanol (n-BUOH)	5	1Methanol Extract	4	1A: Brown	1A = 0.33cm
	Acetic acid	2	2Pet. Ether extract	2	1B: Orange	1B = 0.60cm
	Water (H_2O)	3			1C: Pale yellow	1C = 0.74cm
					1D: Greenish yellow	1D = 0.88cm
					2A: Pale yellow	2A = 0.93cm
					2B: Greenish Yellow	2B = 0.95cm
4	n-Butanol (n-BUOH)	7	1Methanol Extract	2	1A: Orange	1A = 0.60cm
	Acetic acid	2	2Pet. Ether extract	1	1B: Greenish yellow	1B = 0.93cm
	Water (H_2O)	1			2A: Green	2A = 0.95cm
5	n-Butanol (n-BUOH)	6	1Methanol Extract	2	1A: Orange	1A = 0.60cm
	Acetic acid	2	2Pet. Ether extract	1	1B: Greenish yellow	1B = 0.93cm
	Water (H_2O)	2			2A: Green	2A = 0.96cm
6	n-Butanol (n-BUOH)	6	1Methanol Extract	2	1A: Orange	1A = 0.85cm
	Acetic acid	3	2Pet. Ether extract	1	1B: Greenish yellow	1B = 0.93cm
	Water (H_2O)	1			1C: Green	2A = 0.96cm
7	n-Butanol (n-BUOH)	5	1Methanol Extract	2	1A: Orange	1A = 0.61cm
	Acetic acid	1	2Pet. Ether extract	1	1B: Greenish yellow	1B = 0.93cm
	Water (H_2O)	1			2A: Green	2A = 0.85cm
8	n-Butanol (n-BUOH)	5	1Methanol Extract	2	1A: Orange	1A = 0.64cm
	Acetic acid	2	2Pet. Ether extract	1	1B: Greenish yellow	1B = 0.89cm
	Water (H_2O)	3			2A: Greenish Yellow	2A = 0.91cm
9	n-Butanol (n-BUOH)	5	1Methanol Extract	2	1A: Orange	1A = 0.60cm
	Acetic acid	1	2Pet. Ether extract	2	1B: Greenish yellow	1B = 0.90cm
	Water (H_2O)	4			2A: Pale yellow	1C = 0.85cm
					2B: Greenish yellow	2A = 0.92cm
10	n-Butanol (n-BUOH)	5	1Methanol Extract	2	1A: Brown	1A = 0.58cm
	Acetic acid	4	2Pet. Ether extract	1	1B: Greenish yellow	1B = 0.91cm
	Water (H_2O)				2A: Pale Yellow	2A = 0.90cm
11	n-Butanol (n-BUOH)	6	1Methanol Extract	1	1C: Greenish yellow	1A = 0.90cm
	Acetic acid	3	2Pet. Ether extract	1	2A: Green	2A = 0.93cm
	Water (H_2O)					

DISCUSSIONS

The preliminary chemical tests indicated the presence of alkaloids, saponins, flavoniods, tannins and glycosides in the crude extract of the powdered plant substance. The presences of these substances were also confirmed by further phytochemical test done separately on the methanol and petroleum ether extracts. These substances were present both in methanol and petroleum ether extract except for the case of alkaloids which was tested absent in pet. Ether extract. This should however be expected since alkaloids in plants occur as tertiary and quaternary bases. Hence, alkaloids are more soluble in polar solvents than in non-polar or dipolar aprotic solvents. In this case, methanol and water are more polar than petroleum ether [8, 16, 30].

The thin layer chromatographic analysis separated the sample extract into different compounds with different Rf values. The differences in the Rf values of the separated spot is due to the fact that different compounds in a sample mixture travels at different rates due to the different in their attraction to the stationary phase and because of differences in solubility of the solvent system (mobile phase) used. The trend is such that if the stationary phase is more polar than the mobile phase, then the most polar compounds will have the strongest attraction to the stationary phase (resulting to spots with lower Rf values) and the least polar compounds will travel further up the plate (resulting to spots with higher Rf values). By changing the solvent or perhaps using a mixture, the separation of components (measured by Rf value) can be adjusted.

CONCLUSION

This study has screened the phytochemical components of *Cassytha filiformis* using cold solvent extraction and two solvent systems (methanol and petroleum ether) for extraction and standard procedure for testing some phytochemicals. The phytochemical analysis on the plant like alkaloids, saponins, tannins, flavonoids and glycosides and presence of these phytochemicals indicates the therapeutic potentials of this plant. This research also used the TLC method to separate the various constituent of the plant extract. The work has shown that there were at least four different substances (compounds) in the plant extract and that the best eluting solvent system is n-butanol, Acetic acid and water. The solvent system chosen as the best for this work were n-butanol, Acetic acid and water in the ratio 5:2:3. This is because, the mixture separated the plant components into the highest number of spots giving 4 spots and 2 spots for both methanol extract and petroleum ether extract respectively. Also, the spots formed were clear, sharp and well separated from each other. The colours of the sports were equally very noticeable.

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