

Full Length Research Paper

Phytochemical screening of solvent extracts from *Callilepis laureola* plant

Polokuma E. Anderson*, Kojibola Winke and Ereapuduoh B. V

Medical Research Council-Indigenous Knowledge System [Health] Lead Program Unit, P.O. Box 19070, Tygerberg 7505, Francie van Zyl Drive, Parow Valley, Cape Town, South Africa.

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Phytochemical screening of solvent extracts from *Callilepis laureola* plant was studied. Methanol and water extracts, respectively, revealed that phytochemical constituents found in both extracts were phenolics and gums. Reducing sugars were only observed in water extract while phytosterols and flavanoids were only found in methanol extract. Other phytochemical constituents such as saponins, glycosides, anthraquinone, proteins and amino acids were not found in any of the extracts. These phytochemical constituents were detected by colour changes depending on the analysis. Total ash content of the powdered material was found to be 6.90% w/w, water soluble ash was 69.67% w/w and sulphate soluble ash was 16.80% w/w. Thin layer chromatography (TLC) of the plant extracts was also observed and it was concluded that in each extracts, suitable mobile phase must be determined.

Key words: *Callilepis laureola*, phytochemical screening, solvent extracts, ash value, TLC.

INTRODUCTION

Medicinal plants are the mainstay of about 75 to 80% of the world population, mostly in developing countries. In South Africa, many local communities have knowledge of medicinal plants which is still not documented. This knowledge has been transferred from generation to generation. *Callilepis laureola* is a perennial herb which belongs to the Asteraceae family (Gibson, 1975). Its vernacular names are Ox-eye Daisy (English), Wildemargriet (Afrikaans), Mila (SiSwati), Impila (meaning health) /Amafuthomhlaba (meaning oil of the world) (Zulu). *C. laureola* is a medicinal plant which is traditionally used by Zulu people as a multi-purpose remedy to treat stomach problems, tape worm infestations, impotence, cough, and to induce fertility. This medicinal plant was also administered to pregnant women by traditional birth attendants to “ensure the

health of the mother and child” and to facilitate labour as stated by Popat et al. (2001). Bryant (1909) documented that *C. laureola* was very poisonous and has been responsible for several deaths among the Zulu people. In the 1970s, numerous cases of *C. laureola* were reported that this plant induces hepatic and renal toxicity (Wainwright et al., 1977; Seedat et al., 1971; Watson et al., 1979) and these were also reported in media (Anonymous, 1971). Wainwright et al. (1977) conducted a study which described the high incidence of centrilobular liver necrosis in the African population of KwaZulu-Natal. *C. laureola* was identified as the primary causative agent (Wainwright et al., 1977; Bhoola, 1983); during this time there were regular reports of fatal *C. laureola* intoxications (Watson et al., 1979; Steenkamp et al., 1998; Bye et al., 1991, 1992; Grobler et al., 1997). Bye et al. (1991) also wrote a comprehensive review of *C. laureola* related poisoning in South Africa. There was a case involving a 42 year old male who developed hyperkalaemia and acute renal failure due to the ingestion of *C. laureola*. Upon further questioning, the

*Corresponding author. E-mail: anderson.pk@gmail.com

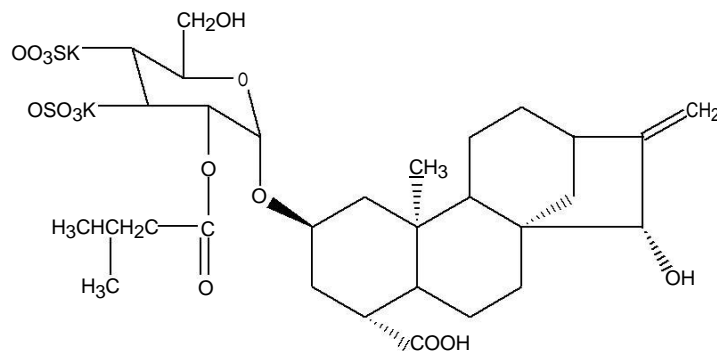


Figure 1. Atractyloside (ATR).

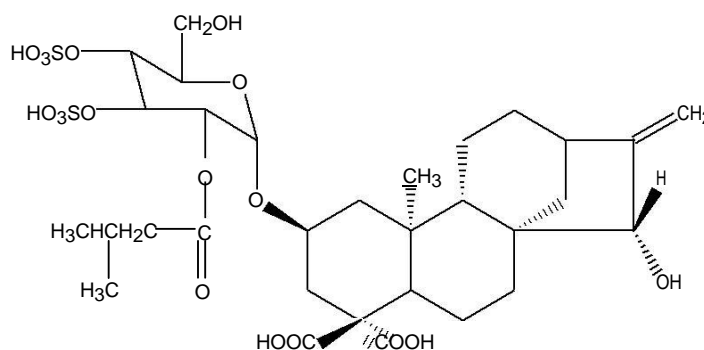


Figure 2. Carboxyatractyloside.

patient said that *C. laureola* was prescribed to him by a traditional healer. The patient did not follow the healer's instructions of preparation. In this particular case, the patient took a dose that was 8 times greater than what the healer prescribed.

Seedat et al. (1971) reported that dosage may play a vital role in the toxicity of *C. laureola*. It is never used in arbitrary doses nor in any but the weakest solution; when swallowed, it must never be allowed to be absorbed. In other words, it is used exclusively in the form of treatment according to Bodestein (1977). There is little doubt that a lack in knowledge and awareness of these strict rules contributed to the numerous cases of *C. laureola*-induced fatalities. Although clinical cases of *C. laureola*-induced toxicity are well documented in the literature, the mechanism by which the plant produces hepatic and renal toxicity is not completely understood. The two major toxic components extracted from the tuber of *C. laureola* are atractyloside (ATR) (Figure 1) and carboxyatractyloside (Figure 2) (Bye et al., 1992; Brookes et al., 1979). Hutchings et al. (1994) and Steenkamp et al. (2004) also confirmed that other compounds isolated include three kaurenoid glycosides, two 6-isovalerates, two new thymol derivatives and a new ketol (Brookes et al., 1979). Further, Steenkamp et al. (2004) showed that some screenings were done but results revealed that

atractyloside and carboxyatractyloside were not responsible for toxicity. The present study investigates the qualitative phytochemical screening and also analyses TLC plates of suitable mobile phase for solvent extracts from *C. laureola* plant.

MATERIALS AND METHODS

Chemicals and reagents

Dichloromethane (DCM), hexane (Hex), ethyl acetate (EtOAc) and methanol (MeOH) were purchased from Merck (Merck, Germany). Deionised and distilled water were prepared by Millipore water system (Milli-QTM, Germany).

Equipment

A superconducting Proton Linac horizontal shaker (Labcon, United State of America) was used for preparing solvent extracts. A Rotary evaporator (Buchi, Switzerland) was used to evaporate organic solvent *in vacuo* while aqueous solvent was frozen and dried using a freeze drier (VirTis genesis wizard 2.0, United Kingdom) to form water extract. Precision balances 220/C/2 (RADWAG Instruments, Germany) were used for

Table 1. Qualitative phytochemical studies of water and methanol extracts from *Callilepis laureolas*.

Constituent	Observation*	
	WT extract	MeOH extract
Physterols	-	+
Flavanoids	-	+
Proteins and amino acids	-	-
Reducing sugars	+	-
Glycosides	-	-
Phenolic acid	+	+
Saponins	-	-
Anthraquinones	-	-
Gums	+	+

* +, present; -, absent; WT - water; MeOH - methanol.

weighing milligram and gram quantities. Finnpiettes (Thermo Labsystems, Canada) were used for spiking volumes.

Plant material and preparation of extracts

Callilepis laureola plant was collected from Kwazulu-Natal in Gbadela district in summer (Gbadela, South Africa). The twigs were washed and dried in an oven (Stainless steel PA Cuthbert and Co., Modderfontein) overnight at 70°C. The dried plant was milled (Donaldson, Torit[®] DCE[®], England) into a fine powder which was used for preparing solvent extracts.

Qualitative phytochemical screening of the extracts

The methanolic and aqueous extracts of *C. laureola* were subjected to various qualitative phytochemical tests to determine the active constituents present in the crude methanolic and aqueous extracts using the procedures of Trease et al. (1989) and Harborne (1985). The phytochemicals tested for were: phytosterols, flavonoids, protein and amino acids, reducing sugars, glycosides, phenolic acid, saponins, anthraquinones, gums.

Physicochemical characteristics

Determination of total ash content

About 2 g of dry powder was accurately weighed and incinerated in a crucible dish at a temperature not exceeding 450°C until free from carbon. It was then cooled to room temperature and then weighed. The % w/w of ash with reference to the air-dried powder was calculated.

Determination of water soluble ash

The total ash was obtained as the above method for preparation of total ash content. The ash was boiled for 5

min with 25 ml water. The insoluble matter was collected using filter paper and washed with hot distilled water and then transferred to a silica crucible. This was then ignited for 15 min at a temperature not exceeding 450°C. The silica crucible and residue were weighed until constant weight was attained.

Determination of sulphated ash

A silica crucible was heated to redness for 10 min then cooled in a desiccator and weighed, after which 2 g plant powder was transferred to the cooled silica crucible and gently ignited until charred. The resulting residue was cooled and moistened with 1 ml of concentrated sulphuric acid. The residue was heated gently until there were no white fumes and this was ignited at 800 ± 25°C until all black particles disappeared. The resulting residue and crucible were cooled and mixed with a few drops of concentrated sulphuric acid and then ignited as before. The resulting residue was cooled and then weighed. This procedure was repeated in duplicate until the results were not different from 0.5 mg.

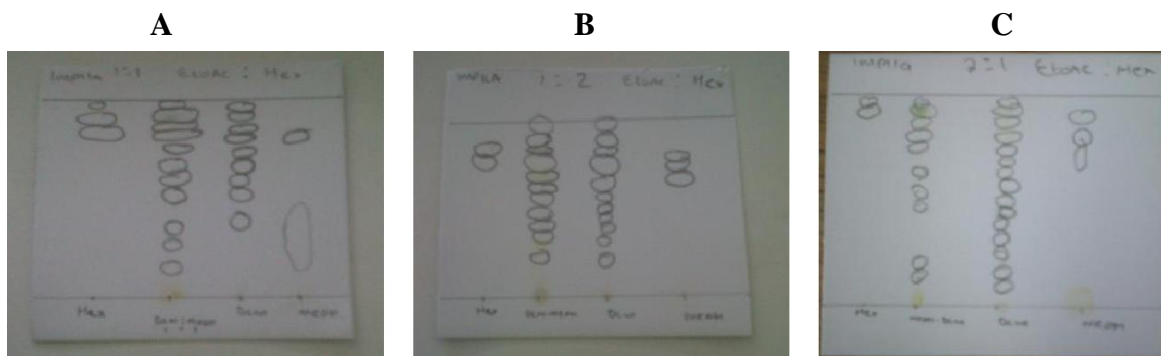
RESULTS AND DISCUSSION

The methanol and water extracts from *C. laureola* were screened for the presence of phytochemicals. The phytochemical test performed was of qualitative type, and from phytochemical investigations, it appears that only phenolic acid and gums were found in both methanol and water extracts (Table 1). Reducing sugars were only observed in water extract and phytosterols and flavanoids were only found in methanol extract. Saponins, glycosides, anthraquinone, proteins and amino acids were the phytochemical constituents which were not found in both extracts (Table 1).

Phytochemical constituents provide an understanding of the biological activities which a particular plant will have. The phytochemical constituents found in *C.*

Table 2. Determination of ash and extractive value from *Callilepis laureola* extract.

Total ash	Ash value (% w/w)	
	Water soluble ash	Sulphate ash
6.9	69.67	16.8

**Figure 3.** Normal TLC plate with: (A) Hexane: ethyl acetate (1:1), (B) Hexane: Ethyl acetate (2:1), (C) Hexane: Ethyl acetate (1:2), where Hex, DCM and MeOH denote the type of extracts.

laureola plant possess the following biological properties: anti-bacterial, anti-inflammatory, cholesterol lowering, act as reducing agents, act as binder, anti-viral, antioxidant and anti-carcinogen, emulsifying of fats. These biological properties are observed when phytochemical constituents are present in plants (Singh et al., 2010; Akpabio et al., 2011; Pascal et al., 2006; Savithramma et al., 2011). Table 2 shows that the total ash of the plant powder was 6.90, 69.67 and 16.80% w/w for total ash, water soluble and sulphate soluble ash, respectively. It appears that this plant powder ash is more soluble to water compared to the other ashes.

Thin Layer Chromatography (Fieser et al., 1992) was used to detect spots from different solvent extracts. Hexane (Hex), Methanol (MeOH), Hex:MeOH- (1:1) and DCM extracts, respectively, were analysed. The following mobile phases were used - Hexane: Ethyl acetate (1:1), Hexane: Ethyl acetate (2:1) and Hexane: Ethyl acetate (1:2). Figure 3 A to C shows that TLC reports that each solvent extract needs to have a specific mobile phase. According to this Figure, hexane extract spots are migrating very fast in all plates which means that there is still a need to find another suitable mobile phase where the spots migrations will be lowered and the resolution of the spots will be efficient. DCM extract seems to have complex spots and this indicates that sequential purification and separation will be needed in all TLC plates. When analysing the Methanol extract, Hexane: Ethyl acetate (1:2) seem to be a mobile phase that shows good resolution, but there must be an improvement by increasing the ethyl acetate and then observing if there will be more separation, or maybe introduce another

solvent which will improve the resolution of the spots.

Conclusion

The qualitative phytochemical screening revealed the chemical constituents which induces the biological activities. Furthermore, the ash values were attained. The TLC plates were analysed to observe the separation of spots from various solvent extracts.

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