

## Methanol Extracts of Medicinal Plants Used for Oral Healthcare in Cameroon

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### Abstract

**Background:** Traditional healers in Cameroon are actively involved in oral healthcare and traditional herbs are used for management of oral diseases. However the analysis of phytoconstituents of medicinal plants used for oral healthcare in Cameroon has not been done. The objective of the study was to determine the phytoconstituents of medicinal plants used for oral healthcare in Cameroon.

**Methods:** Samples of fresh plants used for dental treatment collected from South west and littoral regions of Cameroon that were free from diseases were obtained from the traditional healers. The plants were dried and the dried powder was macerated with intermediate stirring for 48 hours in methanol before filtering with Whatman filter paper. The filtrate was evaporated to dryness of solvent using the rotative evaporator in order to obtain the concentrated extracts of each plant sample. The plant extracts were then qualitatively tested for the presence of phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, steroids and tannins using the standard procedures.

**Results:** All the six (6) plants were found to be rich in at least one of the secondary metabolites. Steroids, terpenoids and flavonoids were found in all the plants samples. Alkaloids were present in *Ageratum conyzoides* and *Carica papaya*. Tannins were present in *Ageratum conyzoides*, *Emilia coccinea*, *Gossypium sp* and *Carica papaya*. Saponins were present in *Ageratum conyzoides*, *Carica papaya* and *Momordica charantia*. Phlobatannins was present in *Emilia coccinea*, *Gossypium sp*, *Carica papaya* and *Spilanthus africana*. Cardiac glycosides were present in *Emilia coccinea*, *Carica papaya*, and *Momordica charantia*. *Carica papaya* contained all these phytochemicals.

**Conclusion:** This study revealed the presence of active secondary metabolites in herbs used for different dental treatment. The clinical trial of phytochemicals on the oral conditions is necessary to determine their pharmacological activity.

**Keywords:** Cameroon; Ethnomedicinal plants; Oral health; Phytochemicals; Traditional healer

### Introduction

Plant chemicals used for medicinal purposes which can be derived from any part of the plant like bark, leaves, fruits, flowers, roots, seeds are largely the secondary metabolites [1]. These secondary metabolites are derived biosynthetically from plant primary metabolites and are not directly involved in the growth, development, or reproduction of plants. They are usually produced in specific groups of plant families or specific tissues, cells or developmental stages throughout plant development [2,3]. The secondary metabolites play a key role in maintaining plant fitness as they function in the protection of plants against microbial (bacteria, fungi and viral) infections, herbivory (slugs and snails, arthropods, and vertebrates), UV radiation, attraction of pollinators and frugivores, allelopathy, and signaling [2,4]. They also serve as drugs, drug precursors, drug prototypes, templates for synthetic modification, and pharmacological probes [4].

Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances [5]. Such phytochemical screening of various plants is reported by many workers [6-8] in other aspects of medicine but not in dentistry.

Green plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations [9].

Medicinal herbs have been used in one form or another under indigenous systems of medicine [10]. It has been mentioned that the complete phytochemical investigations of medicinal plants should be carried out [8], because these secondary metabolites are commercially important and are responsible for medicinal activity of the plant [8,9].

Plants contain secondary metabolites such as alkaloids, flavonoids, steroids, phenolics, terpenes, volatile oils etc. as well as other substance that are important for radical scavenging effects as well as their potential antibacterial, estrogenic and anti-cancer activities [1].

Few reports have been made on the use of medicinal plants in the treatment of dental diseases. These reports have been reported mostly from India and Africa [11-15]. The use of *Argemone maxicana*, *Azadirachta indica* and *Ocimum basilicum* in dental health care has been reported by Singh and Dhakre [11]. Sadangi et al. [15] have reported 10 species of medicinal plants used in the treatment of ear and mouth diseases by the tribal people of Kalandhandi district.

Medicinal plants extracts have been shown to exhibit antibacterial activity against various oral microflora [16-18]. The anti-inflammatory, antioxidants, antibacterial, astringent and other useful properties of several plants and plant parts have reported [19]. These properties are considered valuable in the treatment of diverse dental and oral diseases.

Medicinal plants constitute a significant proportion of traditional medical practices for oral diseases in Cameroon [20]. However the analysis of phytoconstituents of medicinal plants used for oral

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healthcare in Cameroon has not been done. The objective of the study was to determine the phytoconstituents of common medicinal plants used for oral healthcare in Cameroon.

## Materials and Methods

There are many plant materials that have been identified to treat dental problems but specific plants with significant clinical qualities needed phytochemical analysis.

### Selection and identification of plants

Plants analysed during this study were selected specifically based on the frequency of usage by traditional healers and also by specific significant clinical role. Prior to the analysis of these plants, they were identified at the Department of Botany of University of Dchang and Confirmed at the National Herbarium at Yaounde. The following groups of plants were selected to be analyzed;

One plant used for atraumatic tooth extractions, three plants used for dental pain relief, one plant used for tooth bleaching, one plant used for mouth washing/ halitosis. The plants were identified and coded for analytical purposes

**Sample collection:** Samples of fresh plants used for dental treatment in Cameroon that were free from any plant disease were obtained from the traditional healers at the beginning of the rainy season. The plants were dried and one (1) kg of each dried plant was checked for any mould or dust after which was sent to the laboratory for analysis.

**Phytochemical screening:** They were also sterilized on a hot air oven and each material was wrapped with aluminium foil before sterilization.

**Extraction of plant materials:** Different amount of dried powder obtained from each plant material was macerated with intermediate stirring for 48 hours in methanol before filtering. After filtration with Whatman filter paper, the residue was thrown away and the filtrate was evaporated to dryness of solvent using the rotative evaporator in order to obtain the concentrated extracts of each plant sample which was weighed and stored in clean flask in a refrigerator until usage.

**Screening of different plant extracts:** The different plant extracts were qualitatively tested for the presence of phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, steroids and tannins using the standard procedures as previously described [21-24].

### Test for alkaloids

Drangendoff's reagent was used; as such 10 mg of each plant extract in 5 mL of 1% ethanolic HCl and 5 drops of Drangendoff's reagent were added. The formation of orange precipitate incates the presence of alkaloids.

### Test for flavonoids

The method adopted for this study is the one in which 5 mL of diluted ammonia solution was added to a portion of extract dissolved in ethanol, followed by addition of concentrated sulphuric acid. A yellow colouration was observed in each extract, indicating the presence of flavonoids.

### Test for steroids

Two mL of acetic anhydride was added 10 mg ethanolic extract of each sample with 2 mL of concentrated sulphuric acid. The colour changing from violet to blue indicated the presence of steroids.

### Test for terpenoids/terpenes

The Salkowski test was used. Ten milli gram of each extract was dissolved in chloroform, and 4 mL concentrated sulphuric acid was carrefully added to form a layer. A redish brown colouration of the inter-face was formed to show positive result for the presence of terpenoids or terpenes.

### Test for saponins

The persistent frothing test for saponin was used. To 50 mg of each extract was added 5 mL of distilled water. The mixture was vigorously shaken and heated to boil for a stable persistent froth. The frothing was mixed with 4 drops of olive oil and shaken vigorously for the formation of emulsion thus indicating the presence of saponins.

### Test for tannins

About 15 mg of each extract contained in test tube was dissolved in ethanol and 3 drops of Ferric Chloride solution was added. A deep brownish green or a blue-black colouration indicates a positive test for tannins.

### Test for phlobatannins

Deposition of red precipitate when an aqueous extract of each plant sample was boiled with 2% hydrochloric acid was taken as evidence for the presence of phlobatannins.

### Test for cardiac glycosides

The Keller-Killani test was used. About 10 mg of each plant extract dissolved in ethanol was treated with 2 mL of glacial acetic acid containing 2 drops of ferric chloride solution. This was underlayered with 1.5 mL of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides (cardic glycosides). A violet ring appears below the brown ring, while in the acetic acid layer a greenish ring was observed.

## Results

This study revealed the presence of phytochemicals considered as active medicinal chemical constituents in the studied plants. Table 1 shows the total weight percentage yield of the crude extracts of the six plants. Important medicinal phytochemicals such as alkaloids, tannins, saponins, steroids, phlobatannins, terpenoids, flavonoids, and cardiac glycosides were present in the samples. The phytochemical analysis showed that all the six (6) plants are rich in at least one of the secondary metabolites such as alkaloids, tannins, saponins, steroids, phlobatannins, terpenoids, flavonoids, and cardiac glycosides. Steroids,

Plant code	Plant part used	Amount of dry powder used (g)	Amount of extract obtained (g)	Yield (%)
Ag	Aerial part	34	1.5	4.41
Emi	Aerial part	46.5	5.5	11.83
Cot	Leaves	43	3.9	9.06
Cari	Leaves	87	3.4	3.90
Spi	Aerial part	28	2.9	10.35
Mor	Aerial part	6.5	0.4	6.15

Ag: *Ageratum conyzoides* (Asteraceae)

Emi: *Emilia coccinea* (Asteraceae)

Spi: *Spilanthes africana* (Asteraceae)

Cot: *Gossypium sp*, Coton (Malvaceae)

Car: *Carica papaya* (Caricaceae)

Mor: *Momordica charantia* (cucubitaceae)

Table 1: Percentage yeild of extracts.

Plants	Alkaloids	Tannins	Saponins	Steroids	Phlobatannins	Terpenoids	Flavonoids	Cardiac glycosides
Ag	+	+	+	+	-	+	+	-
Emi	-	+	-	+	+	+	+	+
Cot	-	+	-	+	+	+	+	-
Car	+	+	+	+	+	+	+	+
Spi	-	-	-	+	+	+	+	-
Mor	-	-	+	+	-	+	+	+

+: indicates presence of phytochemicals, -: indicates absence of phytochemicals.

Ag: *Ageratum conyzoides* (Asteraceae)

Emi: *Emilia coccinea* (Asteraceae)

Spi: *Spilanthes africana* (Asteraceae)

Cot: *Gossypium sp.*, Coton (Malvaceae)

Car: *Carica papaya* (Caricaceae)

Mor: *Momordica charantia* (Cucurbitaceae)

**Table 2:** Qualitative analysis for phytoconstituents of crude extracts of the six plants.

terpenoids and flavonoids were found in all the plants samples in this study. Alkaloids were present in *Ageratum conyzoides* and *Carica papaya*. Tannins were present in *Ageratum conyzoides*, *Emilia coccinea*, *Gossypium sp* and *Carica papaya*. Saponins were present in *Ageratum conyzoides*, *Carica papaya* and *Momordica charantia*. Phlobatannins were present in *Emilia coccinea*, *Gossypium sp*, *Carica papaya* and *Spilanthes africana*. Cardiac glycosides were present in *Emilia coccinea*, *Carica papaya*, and *Momordica charantia*. *Carica papaya* contained all these phytochemicals (Table 2).

## Discussion

Even though a more recent figure is not available, the WHO has estimated that up to 80% of the population in Africa and the majority of the populations in Asia and Latin America use herbs for their primary healthcare need [25]. In industrialized countries, plant-based traditional medicines or phyto-therapeutics are often termed complementary or alternative medicine (CAM), and their use has increased steadily over the last 10 years [26]. The tradition of herbs use in the treatment of diseases are usually handed from generation to generation in many communities and the repository of the knowledge is predominantly with the traditional healers which is not documented or transferred. The knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances [5]. It has been mentioned that the complete phytochemical investigations of medicinal plants should be carried out because these secondary metabolites are commercially important and are responsible for medicinal activity of the plant [27].

*Argiratum conyzoides* (king plant) is one of the common plants called African panacea or the king of plants in Cameroon because it treats several diseases. This study showed that the plant has many secondary metabolites like alkaloids, tannin, saponins, terpenoids and flavonoids. The result is similar to that carried out by Onuoha and colleagues [27] where all secondary metabolites were present except flavonoids. It is used in Cameroon for calming down pain and for tooth extractions by traditional healers. Its analgesic property is attributed to the ability of alkaloids to inhibit pain in dental caries. The fibrinolytic and hemostatic properties of this plant explain why it is used for tooth extraction. These properties need to be investigated in future. The plant has been found to be effective against septic wounds micro-organisms (*S. aureus* and *E. coli*) [7] and other oral micro-organisms actinomycetes and *P. gingivalis*. It has also been found to have strong cytotoxic effects similar to that of vincristine [28,29]. Huge amount of phenolics and flavonoids present in the plant might be responsible for its promising cytotoxic activity [30,31] and the possible mechanism of cytotoxicity is due to its poisonous effect

on cell mitosis [29]. This cytotoxic property calls for precautions when the plant is being used.

*Emilia coccinea* is used to calm down toothache in Cameroon. The leaves are also eaten raw and can be mixed with guinea corn and lime juice to serve as a remedy for sore throat [32,33]. The leaves are also used for cleaning wounds and the roots for diarrhoea. In Nigeria, the leaves are eaten cooked as salad or spinach and the fresh juice of the leaves is a remedy for sore eyes. According to earlier reports, [34] *Emilia coccinea* is used medicinally for the treatment of syphilis, hernia, gonorrhoea, ulcer, craw-craw, abscesses of the breast, ringworm, lice, measles, cough etc. The bioactivities of *Emilia coccinea* have been confirmed in various laboratories, these include antidiarrhoeal, antimicrobial and fungicidal activity [35] due to its rich constituents of secondary metabolites. In this study the plant extracts contained Tannins, steriods, Phlobatannins, tepenes, Flavonoids and Cardiac glycosides. Alkaloids and saponins were absent. This is consistent with a phytochemical analysis done in Nigeria by Edeoga and colleagues [32] which demonstrated that all phytochemicals were present except Phlobatannins. According to previous studies, the phytochemical screening of *E. coccinea* has revealed the presence of some secondary metabolites including alkaloid, cardiac glycoside [36].

Cotton (*Gosipium barbadense*) leaves are boiled into a hot tea, used for gagging to calm down toothache. It is also used as an adjuvant with *Ageratum conyzoides* for tooth extractions. It contained tannins, steriods, phlobatannins, terpenes and flavonoids. Alkaloids, cardiac glycosides and saponins were absent. Apena and colleagues in Nigeria showed that leaf extracts of *G. barbadense* contained tannins, phlobatannins, cardiac glycosides and flavonoids, but saponins and anthraquinones were absent [37,38]. They also found out that leaf extracts also contained alkaloids such as quinoline, indole and morphine [39,40]. Some species of *Gosipium* have been shown to possess antibacteria, antiinflammatory, wound healing and anti-cancer properties [39,40]. An infusion of the leaf is taken as an antedote for colds and bronchitis and the young shoots pulped for palpitations and as dressings for wounds and in the treatment of systematic diarrhoeas [39]. A number of bioactive triterpenoid and sesquiterpenoid aldehydes compound have been isolated and characterized from this and related species [38].

Whitish latex of *Carica papaya* is applied directly to the affected areas of the toothache to cure toothache while the decoction of its fresh unripe fruits and infusions of its leaves is used for treating mouth sore and oral thrush. *Carica papaya* analysis in this study contained all secondary metabolites tested. This result is similar to other studies [41]. In addition, it contains phytochemicals such as vitamins, enzymes, protiens, alkaloids, lectins, saponins, flavonoids, sterols, minerals and

glycosides [41,42]. In addition, it contains phytochemicals such as vitamins, enzymes, proteins, alkaloids, lectins, saponins, flavonoids, sterols, minerals and glycosides [41,43]. Alkaloids present in *C. papaya* could be responsible for its antibacterial activity and are also used as analgesics and narcotics for pain relief [41].

Chitinase is the anti-fungal enzyme extracted from the plant; the recombinant papaya chitinase has anti-bacterial properties [41]. Capaine, an extract from the seeds and leaves has anti-helmethic properties, boost immunity and also act as cardiac depressant [41]. Flavonoids, glycosides and cardiac glycosides found in the extracts are suggestive of their antioxidant property. Flavonoid glycosides are reported to be antioxidants and used as anti-inflammatories in the treatment of capillary fragility [43] which is applicable to diseases like gingivitis.

The seed and the pulp of *Carica papaya* have been shown to treat more than 20 diseases and studies revealed it's bacteriostatic against common oral microorganisms like *Staphylococcus* spp [41]. The latex of *Carica papaya* has been shown to reduce the growth of *Candida albican* by 60%; the fruits when used as topical ulcers dressing have been found to promote desloughing, granulation and healing [41]. These properties make it suitable for the treatment of mouth sores such as aphthous ulcers.

*Spilanthes africana* is another plant that is widely used by traditional healers in Cameroon. It is also used as a mouth wash for instant treatment of halitosis due to its peppermint taste and for the treatment of minor bone fractures of the teeth and the alveolar bone; when applied directly to the cavity, it alleviates toothache. *Spilanthes acmella* Murr (Compositae) is the well known "toothache plant", also commonly used as a spice. It has a long history of use as a folklore remedy, e.g. for toothache, rheumatism and fever [44]. In this study *Spilanthes africana* was found to contain steroids, phlobatannins, terpenoids, and flavonoids. Sterols are responsible for the antiinflammatory and analgesic properties of this plant. The use of *Spilanthes spp* in the treatment of toothache by direct application by traditional healers had been documented in India [45]. Analgesic and anti-inflammatory activities of different *Spilanthes* species has made it useful for the treatment of toothache, mucositis and sore throat and to relieve pain from boils, cut wounds, and other types of wounds in traditional medicine [45]. Besides, it has been found to be specific against several microorganisms responsible for oro-facial pathologies. *Spilanthes* had been found also to have anti-pyretic, anti-cancer, anti-fungal and anti-oxidant activities [45].

*Momordica charantia* (Cucurbitaceae), the aerial part of the plant is used in Cameroon as an adjuvant in tooth extraction, post extraction management of extraction socket and also for tooth bleaching. The leave extracts is widely used for toothache and post extraction dressing by traditional healers. In the current study, *M.charantia* was found to contain, saponins, steroids, terpenoids, flavonoids and cardiac glycosides. It has been shown to be widely used to treat several diseases because of its anthelmintic, anti-viral, anti-ulcer and antimicrobial activities [46]. Its antibacterial activity has been demonstrated against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [47] which form part of the natural and pathogenic oral flora. It has also been found to exhibit some antifertility, antihyperglycemic [47] and hypoglycemic activities [48]. Its steroids contents have been attributed to its antiglycemic and analgesic activities [47] while terpenes for its antimicrobial and anti-oxidant activities. Extensive studies on the antidiabetic properties of this plant have been done, but more studies are required in the area of oral health care.

## Conclusion

This study revealed that all the medicinal plants studied had huge quantities of secondary metabolites that may be responsible for their medicinal use. More detailed research is needed for these plants because of their multiple uses.

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