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PRELIMINARY PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING ON EXTRACTS OF THE AERIAL PARTS OF *GALIUM SPURIUM* (SUBSPECIES- AFRICANUM)

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ABSTRACT

The common usage of *Galium spp.* in the Ethiopian traditional medicine, as described in few documentations, are generally related to diseases associated with the nervous system, the immune system, infections and inflammation. Preliminary phytochemical and antimicrobial screening on extracts of the aerial parts of *Galium spurium* (subspecies africanum) was done in order to partly rationalize some of the claimed traditional uses of the plant. Results of the preliminary antimicrobial screening on extracts of *G. spurium* showed only weak antibacterial activity. The preliminary phytochemical screening revealed the presence of flavonoids and phenolics compounds. As phenolics and flavonoids are known to display multiple health benefits, the claimed traditional uses of the plant could be justified more through the possible pharmacological effects of these components than its weak antibacterial activities.

INTRODUCTION: The genus *Galium* (Rubiaceae) is composed of perennial herbs or annuals [1]. The root word *Galium* is from the Greek 'gala' meaning "milk" a reference to the ancient use of this genus to curdle milk in order to make cheese. It is also known by the name "bed straw"; a reference to the ancient use of some species of the genus to make beds aroma [2].

In the Ethiopian and Eritrea Flora, only eight species have recognition to represent the genus *Galium*. These are: *G. thunbergianum, G. chlorionanthum, G. aparinoides, G. spurium, G. simense, G. acrophyum, G. scioanum, and G. boreo-aethiopium*. *Galium spurium* (*subspecies africanum*) is mostly distributed in much disturbed areas (grassland or bush land, edge of scrub, roadsides, forest edges, etc) (1000-3000m). It is found in Tigray, Gondar, Gojam, Wello, Arsi, Wellega, Ilbabur, Gamogofa, Sidamo, Bale, and Harar regions of Ethiopia. It is also found in west Eritrea, Somalia, and also distributed starting from Sudan south to south Africa [1]. Like most *Galium spp*, *G. spurium* is known in Ethiopia by the vernacular names: 'Ashkit' in Amaharic, 'Tsin-ee' or 'Tsegwego' in Tigrigna and 'meeteeto'or 'mexxenee' in oromigna [1, 3].

Many *Galium* species has long been used for a number of medicinal purposes. Generally, there are limited reports about Ethiopian traditional medicine; and few reports mention that *Galium spp.,* especially *G. simense*, are traditionally used usually in combination with other plants and rarely alone.

Such uses include: use in 'Megagna' and insanity; as tinae against tapeworm; in eye diseases (for crying eyes), earache (as eardrops); as haemostatic agent; and in wound conditions [4, 5]. Megagna' is a local word used to express CNS disorders characterized by epileptic conditions; the causes of which are believed to be associated with demonic or evil consequences [4]. Generally, these folklore uses can be interpreted in to scientific term through pharmacological manifestations that mainly target the nervous system, the immune system, anti-infective, antioxidant, and anti-inflammatory activities.

In this study, the antimicrobial (antibacterial) and phytochemical screening on extracts of *Galium spurium* (*subspecies africanum*) were done so as to partly rationalize some of the claimed traditional uses of the plant.

MATERIALS AND METHODS:

Plant Material: The whole herb was collected in April and May from Addis Ababa University (Arat killo campus and FBE campus). The developmental stage of the collected herb was about the time of flowering and fruiting, but before maturation of fruits. The root and aerial parts of the herb were separately chopped while fresh, air-died, powdered and reserved for extraction. The plant material was authenticated in the National Herbarium, Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia.

Extraction and preparation of Solvent Fractions: Enough amount of powdered aerial part was macerated overnight, in a flask, using 80% aqueous methanol. After filtration the marc was decocted for one hour using the same solvent, cooled and filtered. The two filtrates were mixed and concentrated under reduced pressure; and the concentrate was transferred in to an evaporating dish and dried in an air-drier oven. Portion of the dried crude extract was re-suspended in hydro alcoholic solvent (50% aqueous methanol). This hydro alcoholic suspension was washed with equal volumes of petroleum ether, diethyl ether and chloroform; each three times. The three portions of each was collected, mixed, concentrated, and dried in an air-drier oven. The washed hydro alcoholic fraction was similarly dried. The crude extract and solvent fractions were reserved for preliminary phytochemical and antibacterial screening.

Preliminary Phytochemical Screening: Preliminary phytochemical screening was performed using common chemical test procedures and chromatographic techniques [thin layer chromatography (TLC) and paper chromatography (PC)] as described in the manual by Debela[6] and in the book by Trease and Evans [7]. Screening test was made for alkaloids, flavonoids, coumarins, anthraquinone derivatives, tannins and phenolic compounds.

Antimicrobial studies:

1. **Microorganisms:** *Staphylococcus spp*., *Streptococcus spp., Pseudomonas spp., Escherichia coli,* and *Klebsiella spp*. were the bacteria used for the antibacterial activity study. All the microorganisms were clinical isolates and were obtained from the Bacteriology laboratory in Black Lion Hospital, Addis Ababa University.

2. **Media and Test Extracts:** Agar Media were prepared according to manufacturer direction by Bacteriology laboratory personnel in Black Lion Hospital. Blood agar was used as growth media for *Streptococcus* sp and Nutrient agar media was used to grow *Staphylococcus*, *Pseudomonas*, *Klebsiella* and *E. coli*. 100mg of petroleum ether fraction and 80% aqueous methanol extract were dissolved, in separate flasks, using 10ml methanol to prepare the test extracts.

3. **Preliminary Antibacterial Testing:** The well diffusion method was used to test the antibacterial activities of the extracts. 100µl of each of the solution mentioned above were applied into the formed well on the agar medium. Application of solution was made after the media had been inoculated with the particular bacteria. Equal volume of the pure solvent (methanol) has also been applied in the same manner as the sample solutions to serve as a negative control and standards of antibiotics (penicillin in case of *streptococcus* and norfloxacillin in case of *Staphylococcus, Pseudomonas, E. coli* and *Klebsiella*) in the form of disks were used as a positive control. Zones of inhibition were measured using ruler after 24hrs incubation at 37^{o}C.

RESULTS AND DISCUSSIONS: The developmental stage of the collected herb was chosen at about the time of flowering and before the maturing of the fruits as this time is believed to be the time when photosynthesis is very active and produce highest yield of constituents [8].

The results of preliminary phytochemical screening showed the presence of flavonoids and phenolic compounds, but tannins, alkaloids and anthraquinone derivatives were not detected in the specific tests used.

As with the chemical test, the TLC and PC analysis revealed the presence of flavonoids. The TLC analysis also revealed the presence of fluorescing compounds. As can be seen from **Table 1**, the results of preliminary antimicrobial tests are not promising.

Of course, there exist some activities against *streptococcus sp.* and *Klebsiella sp.* by the petroleum ether fraction and weak activity against *pseudomonas sp.* by the 80% aqueous methanol extract; but no activity was shown against *Staphylococcus sp.* and *E. coli*. Thus, the overall result was not promising to perform or recommend further tasks related to antimicrobial activity.

Hence, the claimed traditional use of the plant could be verified by other possible pharmacological effects than antibacterial effects. Indeed, it is reported that ethanol extract from the aerial parts of *Galium spurium* showed anticonvulsant activity [9]; which is an indication for possible effects of the plant on the nervous system and hence its traditional use in epileptic conditions and insanity can be reasonable.

TABLE 1: RESULTS OF ANTIBACTERIAL TESTS ON VARIOUS EXTRACTS OF THE AERIAL PARTS OF *GALIUM SPURIUM*

Bacterial type	Zone of inhibition as measured in diameter (mm)			
	Pet-ether	80% MeOH	N	P
Staphylococcus spp.	0	0	0	33
Streptococcus spp.	13.5	0	0	49
Pseudomonas spp.	11	14.5	10.7	38.5
E. coli	0	0	0	40
Klebsiella spp.	15	0	0	36

(Pet-ether: Petroleum ether fraction; 80% MeOH: 80% aqueous methanol extract; N: Negative control (methanol); P: Positive control (penicillin for *streptococcus spp.* and norfloxacillin for the other four species)

Phytoconstituents reported in *Galium spurium* include some iridoids (asperuloside, asperulosidic acid, asperulosidic acid methyl ester, deacetyl-asperulosidic acid, aucubin and monotropein), flavonoids (rutin, quercetin, iso-quercitrin, isoorientin, quercetin and kaempferol derivatives), phenolic acids (caffeic acid and chlorogenic acid), antroquinone derivatives and the tritrpene ursolic acid [9].

As was noted above, the preliminary phytochemical screening revealed the presence of flavonoids and phenolic compounds, which in turn are receiving greater attention because of their multiple health benefits. For example, literature reports the anti-inflammatory, immunomodulatory and antioxidant effects of caffeic acid [10]. Caffeic acid exerts a protective effect in traumatic brain injury, which at least is associated with its antioxidant effects [11]. Both chlorogenic acid and caffeic acid are expected to show similar effects as chlorogenic acid is hydrolyzed into caffeic acid in the intestine [12].

The flavonoids quercetin and rutin have antioxidant and neuroprotective effects [13, 14, 15]. Rutin has antidepressant-like effect [16], anti-inflammatory [17, 18], and protective effect against hepatototoxicity and memory impairment [18].

Ursolic acid has numerous pharmacological effects including anti-inflammatory, hepatoprotective, anti-oxidant [19, 20], and neuroprotective [21]. Therefore, it seems that the claimed traditional uses of *G. spurium* could be better justified by other possible activities such as antioxidant, anti-inflammatory and through modulatory effects on the nervous system and immune system than antibacterial activities.

CONCLUSIONS: The traditional uses of *Gallium spp*. in Ethiopia indicate its probable anti-infective, antioxidant, anti-inflammatory activities; and possible effect on the nervous system and immune system. Results of preliminary antimicrobial screening on extracts of *G. spurium* showed only weak antibacterial activity against limited bacterial strains.

The preliminary phytochemical screening revealed the presence of flavonoids and phenolic compounds. Literature reports confirm the presence of flavonoids (e.g. quercetin and rutin) and phenolic acids (e.g. caffeic acid and chlorogenic acid), which in turn are reported to display numerous pharmacological effects including anti-inflammatory, antioxidant, immuno-modulatory, and neuroprotective.

Thus, it seems that the claimed traditional uses of *G. spurium* could be better justified through its potential activities such as antioxidant, anti-inflammatory and through modulatory effects on the nervous system and immune system than its weak antibacterial activities.

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