Academic Journal of Cancer Research 5 (2): 31-39, 2012
ISSN 1995-8943

DOI: 10.5829/idosi.ajcr.2012.5.2.66210

Phytochemical Analysis of Phenol and Flavonoid in Eight Selected Medicinal Herbs Used for the Treatment of Diabetes, Malaria and Pneumonia in Kisii, Kenya

[1]Moses A.G. Maobe, [1]Leonard Gitu, [1]Erastus Gatebe and [2]Henry Rotich

[1]Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology,
P.O. Box 62000, Nairobi, Kenya
[2]Kenya Bureau of Standards, P.O Box 54974, Nairobi

Abstract: Medicinal herbs are an important source of phytochemicals that offer traditional medicinal treatment of various ailments. Flavonoids are phytochemical compounds that provide protection against ultraviolet radiation, pathogens and herbivores to herbs. Flavonoids have effect on human nutrition and health as antioxidant and chelating compounds. Phenols are phytochemical compounds that function in nutrient uptake, protein synthesis, enzyme activity, photosynthesis; structural components and allelopathy in herbs. The phenolic compounds, have biological and pharmacological properties especially their antimicrobial activity, antiviral, anti-inflammatory and cytotoxic activity, antimutagenic and anticarcinogenic activities. In Kisii region, southwest Kenya, amongst the herbs used as phytomedicines for the treatment of diabetes, malaria and pneumonia are *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis Peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica.* A study was carried out on these herbs in the year 2011 to 2012. The objective was to determine the phytochemical compounds of flavonoids and phenolic acids present in the eight selected medicinal herbs by thin layer chromatography (TLC) method. In the study, leaf samples of the selected herbs growing in the ecological conditions of the Kisii region were collected, washed, air-dried and milled. The samples were extracted with hexane, dichloromethane, ethyl acetate and ethanol solvents. Thin layer chromatography was used to determine the phytochemical compounds of flavonoids and phenolic acids present in the hexane, dichloromethane, ethyl acetate and ethanol extracts of the medicinal herbs. Results of the retention factor (R_f) obtained were compared with that of the standard TLC system. The TLC finger print of the eight tested herbs showed the band having R_f = 0.67, 0.24, 0.88, 0.83, 0.73, 0.67, 0.34, 0.5, 0.13, 0.74, 0.35, 0.85, 0.2, 0.23, 0.75, 0.7, 0.7, 0.34 which was equivalent to Flavanone, Naringenin, Flavone, 3-Hydroxyflavone, 6-Hydroxyflavone, 6'-Hydroxyflavone, 7-Hydroxyflavone, 3,6-Dihydroxyflavone, 3,7-Dihydroxyflavone, Morin, Chrysin, Quercetin, Galangin, Apigenin, Kaempferol, *O*-Coumaric acid, *p*-Coumaric acid, Caffeic acid and Ferulic acid, respectively. Flavonoid and phenolic acid compounds are present in all the analysed selected eight medicinal herbs. The number of phytochemical compounds isolated by TLC and identified ranges from one to nine. The known flavonoid compounds in *Leonotis nepetifolia* are five*, Bidens pilosa* are four*,* Urtica *dioica, Senna didymobotrya and Toddalia asiatica* are three, *Warburgia ugandensis and Physalis peruviana* are two and none in *Carissa spinarum,* respectively. Known phenolic acid compounds in *Bidens pilosa* are nine*, Leonotis nepetifolia* are seven*, Senna didymobotrya* are six, *Physalis peruviana* are four*, Warburgia ugandensis* are three*, Toddalia asiatica* are two and one in *Carissa spinarum* and *Urtica dioica.* Further studies are needed to identify the unknown flavonoids and Phenolic acids in the analysed herbal extracts.

Key words: Medicinal Herbs · Pytochemicals · Phenol · Flavonoid · TLC

Corresponding Author: Moses A. G. Maobe, Department of Chemistry,
Jomo Kenyatta University of Agriculture and Technology,
P.O. Box 62000, Nairobi, Kenya.

INTRODUCTION

Plants have been associated with the human health from time immemorial and they are the important source of medicines since human civilization [1]. Plants still remain one of the major sources of drugs in modern as well as in traditional systems of medicine. In the Kisii region, southwest Kenya, the leave decoction of *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica*, are used for the treatment of diabetes, malaria and pneumonia [2]. Plant secondary metabolites are important sources of many food ingredients and phytochemicals [3]. Plants produce several secondary metabolite compounds including alkaloids, cyanogenic glycosides, glucosinolates, flavanoids, saponins, steroids and terpenoids to protect themselves from the continuous attack of naturally occurring pathogens, insect pests and environmental stresses [4, 5]. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties [6]. Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemicals can protect humans against diseases [7]. There are many phytochemicals in herbs and each works differently. These phytochemicals have various health benefits such as antioxidant, anti-microbial, anti-inflammatory, cancer preventive, anti-diabetic and anti-hypertensive effects [8, 9]. According to Pawar *et al*, [10], the physiological action on the human body can be determined by these phytochemicals. Many of the plant extracts have proven to possess pharmacological action [10] due to the presence of various phytochemicals. The flavonoids are known as phytochemical compounds that provide protection against ultraviolet radiation, pathogens and herbivores [11]. Plants are able to synthesize a multitude of organic molecules or phytochemicals, referred to as “secondary metabolites”. These molecules play variety of role in the life span of plants, ranging from structural ones to protection. Phenolic compounds are regarded as one such group that is synthesized by plants during development and in response to conditions such as infection, wounding, UV radiation [12, 13]. Phenols are associated with diverse functions, including nutrient uptake, protein synthesis, enzyme activity, photosynthesis; structural components and allelopathy [14]. Phenolics show an array of health promoting benefits in human health. The phenolic compounds, have biological and pharmacological properties especially their antimicrobial activity [15-19], antiviral, anti-inflammatory and cytotoxic activity, the antimutagenic and anticarcinogenic activities [15- 19]. The medicinal herbs are enriched with phenolic compounds and bioflavonoids that have excellent antioxidant properties [20]. Phenolics are active in curing kidney and stomach problems as well as helpful as anti-inflammatory in action [21]. Flavonoids are common within the plant kingdom. More than 5000 flavonoids have been identified in nature [22]. They function as stress protectants in plant cells by scavenging reactive oxygen species produced by the photosynthetic electron transport system. Due to UV-absorbing properties, flavonoids protect plants from the UV radiation of the sun and scavenge UV-generated reactive oxygen species. Flavonoids are considered as important components in the human diet, although they are generally considered as non-nutrients. Flavonoid intake can range between 50 and 800 mg per day, depending on the diet consumption. Flavonoids has been considered to have effect on human nutrition and health as it show antioxidant activity and their mechanism of action are through scavenging or chelating process [23]. It has been recognized that phenolic compounds are a class of antioxidant compounds which act as free radical terminators. These compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for antioxidant effect in the plants [24]. Pharmacological activities of a drug are contributed by the presence of secondary metabolites. In the class, compounds such as alkaloids, saponins, tannins, anthraquinones and flavonoids are known to have activity against several pathogens and therefore could suggest their traditional use for the treatment of various illnesses [25]. Thin layer chromatography is a standard technique for separating organic compounds [26]. Thin layer Chromatography is mainly used as an inexpensive method for separation and determination of the chemical constituents in the eight selected traditional medicinal herbs [26]. The use of medicinal plants in a traditional way is becoming revitalized over the world because modern medicine is becoming more widespread but at a high cost. In rural area, medicinal herbs are useful in certain cases, like surgery [27]. Medicinal herbs that are used in postpartum herbal bath and food supplement may be responsible for antioxidation action, anti-inflammatory and antimicrobial activity on target organs of mother [27].

The objective of this study was to determine the phytochemical compounds of flavonoids and phenolic acids present in eight selected traditional medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii Region, Southwest Kenya by thin layer chromatography (TLC) method.

MATERIALS AND METHODS

Sample Collection and Preparation: In this study the leaves of the *Carissa spinarum, Urtica dioica, Warburgia ugandensis, Senna didymobotrya, Physalis peruviana, Bidens pilosa, Leonotis nepetifolia* and *Toddalia asiatica* were collected from Kisii region, southwest Kenya. The verification of the herbal species was done by the Botanist; Egerton University. The leaves of the validated medicinal herbs were then collected from their site in Kisii region and air-dried for twelve weeks to obtain constant weight. The dried sample was cut into smaller pieces and then ground into fine particles with a grinder at the Department of Food Science and Technology, Faculty of Science, Jomo Kenyatta University of Agriculture and Technology. The powdered sample was bagged in black plastic bags and stored in an air-tight container for further work.

Preparation of Extracts: In order to perform phytochemical analysis, thirty grams of dry ground plant leaves of each of the eight herbs was extract with hexane, dichloromethane, ethyl acetate and ethanol solvents, shaken for 5 hours then kept at room temperature for 24 hours in closed 250 ml conical flask containers. The extraction process was repeated 3 times (extraction for 3 days). Then the extracts were filtered under vacuum and concentrated at reduced pressure using a rotary evaporator. The dried extracts were kept in the refrigerator at 4 °C until use.

Phytochemical Analysis: The identification of the chemical constituents; flavonoids, polyphenolic compounds and phenolic acids in hexane, dichloromethane, ethyl acetate and ethanol extracts of the eight herbs was carried out according to the standard method (Panyaphu *et al.*, 2012).

Thin Layer Chromatography: Thin layer chromatography was performed on the crude extracts of hexane, dichloromethane, ethyl acetate and ethanol using thin layer chromatographic plates according to (Marica *et al*., 2004). About 0.5g each of the crude extract was dissolved in 2ml of hexane, dichloromethane, ethyl acetate and ethanol in different beakers. In each case 1cm was measured from the base of the TLC plate, marked with a pencil and labelled. Capillary tube was used to spot the plates with the crude extract. Small quantities of the concentrated solutions were collected with capillary tube by dipping it in the solution. They were then used to spot the plates; three spots were made on each plate. Labelling of the samples was done with a pencil. The developing tank was prepared by measuring 15ml of hexane and poured into a chromatank (with a 20cm x 20cm base area). The plates were placed in a chromatank and covered, ensuring that the solvent was just below the spots. Apparatus was placed on a level surface for the solvent to rise. The plate was removed after about two hours when the solvent had risen close to the top edge, marking the distance travelled by solvent with a pencil. It was then dried at room temperature. The dried plate was then placed in a container, with iodine vapour to develop the spots on the plates. The spot moved by solvents and shape of spots were also marked out with a pencil. The distances moved by the solvents and the spots were measured, in millimetres, with a rule. The retention factors of the samples were then determined. The retention factor, R_f is defined as the distance travelled by the compound divided by the distance travelled by the solvent.

$$\text{I.e. } R_f = \frac{\text{distance travelled by the compound}}{\text{Distance travelled by the solvent}}$$

The same procedure was repeated for the crude extracts of dichloromethane, ethyl acetate and ethanol using the following solvent systems: hexane: dichloromethane, ethyl acetate: ethanol (1:1), hexane: dichloromethane (9:1), hexane: dichloromethane (3:1), dichloromethane: ethyl acetate (9:1), dichloromethane: ethyl acetate (3:1), dichloromethane: ethyl acetate (1:3), ethyl acetate: ethanol(9:1), ethyl acetate: ethanol(3:1), ethyl acetate: ethanol(1:1), ethyl acetate: ethanol(1:3).

RESULTS AND DISCUSSION

Results obtained on distances travelled by solvent front and extracts on performing the thin layer chromatography were analysed and the R_f values are displayed and discussed in this section. The TLC analysis in various solvent systems for each solvent type revealed the presence of spots that range from one to maximum of ten (Tables 1- 4). Each spot is presumably due to a pure natural product or phytochemical. Each also has a specific R_f value. The larger the R_f value, the lower the polarity of natural products or phytochemical.

Carissa Spinarum: Results obtained indicate that in comparison with standards R_f value in similar conditions, one spots in the chromatogram of t*Carissa spinarum* ethyl acetate extract was equal to the standards (R_f values = 0.20). These results indicated that t*Carissa spinarum* may contain Apigenin (Table 5). However, there are unknowns that need to be identified in further research.

Table 1: Showing the results of the TLC for hexane extracts

Hexane extracts	Solvent system	Number of spots visible by UV
Carissa spinarum	Hexane: dichloromethane, (50:50 v/v)	1
Urtica dioica	Hexane: dichloromethane, (50:50 v/v)	1
Warburgia ugandensis	Hexane: dichloromethane, (50:50 v/v)	4
Senna didymobotrya	Hexane: dichloromethane, (50:50 v/v)	2
Physalis peruviana	Hexane: dichloromethane, (50:50 v/v)	7
Bidens pilosa	Hexane: dichloromethane, (50:50 v/v)	10
Leonotis nepetifolia	Hexane: dichloromethane, (50:50 v/v)	6
Toddalia asiatica	Hexane: dichloromethane, (50:50 v/v)	2

Table 2: Showing the results of the TLC for dichloromethane extracts

Dichloromethane extracts	Solvent system	Number of spots visible by UV
Carissa spinarum	Dichloromethane; Hexane, (90:10 v/v)	3
Urtica dioica	Dichloromethane; Hexane, (90:10 v/v)	3
Warburgia ugandensis	Dichloromethane: Hexane, (90:10 v/v)	5
Senna didymobotrya	Dichloromethane: Hexane, (90:10 v/v)	4
Physalis peruviana	Dichloromethane: Hexane, (90:10 v/v)	3
Bidens pilosa	Dichloromethane/Hexane, (90:10 v/v)	4
Leonotis nepetifolia	Dichloromethane: Hexane, (90:10 v/v)	3
Toddalia asiatica	Dichloromethane: Hexane, (90:10 v/v)	3

Table 3: Showing the results of the TLC for ethyl acetate extracts

Ethyl acetate extracts	Solvent system	Number of spots visible by UV
Carissa spinarum	Ethyl acetate/dichloromethane, (90:10 v/v)	2
Urtica dioica	Ethyl acetate/dichloromethane, (90:10 v/v)	1
Warburgia ugandensis	Ethyl acetate/dichloromethane, (90:10 v/v)	1
Senna didymobotrya	Ethyl acetate/dichloromethane, (90:10 v/v)	7
Physalis peruviana	Ethyl acetate/dichloromethane, (90:10 v/v)	2
Bidens Pilosa	Ethyl acetate/dichloromethane, (90:10 v/v)	4
Leonotis nepetifolia	Ethyl acetate/dichloromethane, (90:10 v/v)	4
Toddalia asiatica	Ethyl acetate/dichloromethane, (90:10 v/v)	4

Table 4: Showing the results of the TLC for ethanol extracts

Ethanol extracts	Solvent system	Number of spots visible by UV
Carissa spinarum	Ethanol/Hexane, (90: 10)	1
Urtica dioica	Ethanol/Hexane, (90: 10)	3
Warburgia ugandensis	Ethanol/Hexane, (90: 10)	2
Senna didymobotrya	Ethanol/Hexane, (90: 10)	4
Physalis peruviana	Ethanol/Hexane, (90: 10)	3
Bidens pilosa	Ethanol/Hexane, (90: 10)	3
Leonotis nepetifolia	Ethanol/Hexane, (90: 10)	3
Toddalia asiatica	Ethanol/Hexane, (90: 10)	3

Chemical constituents: The R_f value obtained was compared with R_f value of standards / TLC System in the similar conditions to identify the flavonoids and phenolic acids present in the eight herbs according to Panyaphu [27].

Table 5: R_f Value of Flavonoids, Phenolic acids in the extracts of*Carissa spinarum*

St*Carissa spinarum*.	R_f values			
TLC System	Hexane extracts	Dichloromethane extracts	Ethyl acetate extracts	Ethanol extracts
Apigenin	-	-	0.20	-

This table was adapted from Marica *et al*., 2004

Solvent systems used in chromatographic system

Hexane extract = Hexane: dichloromethane, (50:50 v/v)

Dichloromethane extract = Dichloromethane: Hexane, (90:10 v/v)

Ethyl acetate extract = Ethyl acetate: dichloromethane, (90:10 v/v)

Ethanol extract = Ethanol: Hexane, (90:10)

Table 6: R_f Value of Flavonoids, Phenolic acids in the extracts of *Urtica dioica*

S *Urtica dioica* tst	R_f values			
Stand TLC Systems	Hexane extracts	Dichloromethane extracts	Ethyl acetate extracts	Ethanol extracts
Flavanone	-	-	-	0.67
3-Hydroxyflavone	-	-	-	0.83
6-Hydroxyflavone	-	-	-	0.67
Apigenin	-	-	-	0.67

This table was adapted from Marica *et al*., 2004
Solvent systems used in chromatographic system
Hexane extract = Hexane: dichloromethane, (50:50 v/v)
Dichloromethane extract = Dichloromethane: Hexane, (90:10 v/v)
Ethyl acetate extract = Ethyl acetate: dichloromethane, (90:10 v/v)
Ethanol extract = Ethanol: Hexane, (90:10)

Table 7: R_f Value of Flavonoids, Phenolic acids in the extracts of *Warburgia ugandensis*

Warburgia ugandensis C	R_f values			
TLC Systems	Hexane extracts	Dichloromethane extracts	Ethyl acetate extracts	Ethanol extracts
Naringenin	-	0.24	-	-
Flavanone	-	-	-	0.85
Morin	-	0.13, 0.15	0.23	-
Galangin	-	-	-	0.85
Kaempferol	-	-	0.23	-

This table was adapted from Marica *et al*., 2004
Solvent systems used in chromatographic system
Hexane extract = Hexane: dichloromethane, (50:50 v/v)
Dichloromethane extract = Dichloromethane: Hexane, (90:10 v/v)
Ethyl acetate extract = Ethyl acetate: dichloromethane, (90:10 v/v)
Ethanol extract = Ethanol: Hexane, (90:10)

Urtica Dioica: Results obtained indicate that in comparison with standards R_f value in similar conditions, two spots in the chromatogram of *Urtica dioica* t ethanol extract was equal to the standards (R_f values = 0.67 and 0.83 respectively).These results indicated that *Urtica dioica* may contain Flavanone, 3-Hydroxyflavone, 6-Hydroxyflavone and Apigenin (Table 6). However, there are unknowns that need to be identified in further research.

Warburgia Ugandensis: Results obtained show that in comparison with standards R_f value in similar conditions, three spots in the chromatogram of dichloromethane extract, one spot in the chromatogram of ethyl acetate and t ethanol extract of *Warburgia ugandensis* was equal to the standards (R_f values = 0.13, 0.15, 0.23, 0.24 and 0.85 respectively).These results indicated that *Warburgia ugandensis* may contain Naringenin, Flavanone, Morin, Kaempferol and Galangin (Table 7). However, there are unknowns that need to be identified in further research.

Senna Didmobotrya: Results obtained show that in comparison with standards R_f value in similar conditions, one spot in the chromatogram of hexane and dichloromethane extract, five spots in the chromatogram of ethyl acetate and t three spots in the chromatogram of t ethanol extract of *Senna didmobotrya* was equal to the standards (R_f values = 0.13, 0.20, 0.5. 0.70, 0.74 and 0.82 respectively). These results indicated that *Senna didmobotrya* may contain 3,7- Dihydroxyflavone, 3-Hydroxyflavone, 7-Hydroxyflavone, Kaempferol, Morin, Apigenin, Chrysin, *p*-Coumaric acid and Ferulic acid (Table 8). However, there are unknowns that need to be identified in further research.

Physalis Peruviana: Results obtained show that in comparison with standards R_f value in similar conditions, five spots in the chromatogram of hexane, one spot in the chromatogram of dichloromethane and ethyl acetate extract and t two spots in the chromatogram of t ethanol extract of *Physalis peruviana* was equal to the standards (R_f values = 0.12, 0.13, 0.20, 0.34, 0.82 and 0.83 respectively). These results indicated that *Physalis peruviana* may contain 3, 6-Dihydroxyflavone, 3-Hydroxyflavone, Morin, *p*-Coumaric acid, Caffeic acid and Apigenin (Table 9). However, there are unknowns that need to be identified in further research.

Table 8: R_f Value of Flavonoids, Phenolic acids in the extracts of selected herbal species

Senna didmobotrya /	R_f values			
StandTLC Systems	Hexane extracts	Dichloromethane extracts	Ethyl acetate extracts	Ethanol extracts
3,7- Dihydroxyflavone	0.5	-	-	0.70
3-Hydroxyflavone	-	-	0.82	-
7-Hydroxyflavone	-	-	-	0.70
Morin	-	0.13	-	-
Apigenin	-	-	0.20	-
Chrysin	-	-	0.74	-
Kaempferol	0.5	-	-	-
p-Coumaric acid	-	-	-	0.70
Ferulic acid	-	-	-	0.70

This table was adapted from Marica *et al*., 2004

Solvent systems used in chromatographic system

Hexane extract = Hexane: dichloromethane, (50:50 v/v)

Dichloromethane extract = Dichloromethane: Hexane, (90:10 v/v)

Ethyl acetate extract = Ethyl acetate: dichloromethane, (90:10 v/v)

Ethanol extract = Ethanol: Hexane, (90:10)

Table 9: R_f Value of Flavonoids, Phenolic acids in the extracts of*Physalis peruviana*

St*Physalis peruviana*	R_f values			
TLC Systems/ T	Hexane extracts	Dichloromethane extracts	Ethyl acetate extracts	Ethanol extracts
3,6-Dihydroxyflavone	0.34	-	-	-
3-Hydroxyflavone	-	-	-	0.82, 0.83
Morin	0.13	0.12	-	-
Apigenin	-	-	0.20	-
p-Coumaric acid	0.34	-	-	-
Caffeic acid	0.34	-	-	-

This table was adapted from Marica *et al*., 2004

Solvent systems used in chromatographic system

Hexane extract = Hexane: dichloromethane, (50:50 v/v)

Dichloromethane extract = Dichloromethane: Hexane, (90:10 v/v)

Ethyl acetate extract = Ethyl acetate: dichloromethane, (90:10 v/v)

Ethanol extract = Ethanol: Hexane, (90:10)

Table 10: R_f Value of Flavonoids, Phenolic acids in the extracts of *Bidens pilosa*

Bidens pilosa	R_f values			
TLC Systems/ T	Hexane extracts	Dichloromethane extracts	Ethyl acetate extracts	Ethanol extracts
Flavanone	0.75	-	-	-
Flavone	-	-	-	0.85
6-Hydroxyflavone	0.75	-	-	-
7-Hydroxyflavone	0.42	0.42	0.47	0.70
3,7-Dihydroxyflavone	0.5	-	0.47	0.70
Morin	0.13	0.13	-	-
Apigenin	0.20	-	-	-
Quercetin	0.35	-	-	-
Galangin	-	-	-	0.85
Apigenin	-	-	0.20, 0.47	-
Kaempferol	0.5	-	0.47	-
O-Coumaric acid	0.75	-	-	-
p-Coumaric acid	0.75	-	0.47	-
Ferulic acid	-	-	-	0.70

This table was adapted from Marica *et al*., 2004

Solvent systems used in chromatographic system

Hexane extract = Hexane: dichloromethane, (50:50 v/v)

Dichloromethane extract = Dichloromethane: Hexane, (90:10 v/v)

Ethyl acetate extract = Ethyl acetate: dichloromethane, (90:10 v/v)

Ethanol extract = Ethanol: Hexane, (90:10)

Table 11: R_f Value of Flavonoids, Phenolic acids in the extracts of *Leonotis nepetifolia*

Leonotis nepetifolia	R_f values			
TLC Systems	Hexane extracts	Dichloromethane extracts	Ethyl acetate extracts	Ethanol extracts
Naringenin	0.73	-	-	-
Flavone	-	-	0.88	0.87
6-Hydroxyflavone	0.73	-	-	-
3,6-Dihydroxyflavone	0.34	-	-	0.72
3,7-Dihydroxyflavone	0.5	-	-	-
Morin	-	0.13	-	-
Galangin	-	-	-	0.72
Apigenin	-	-	0.20	-
Kaempferol	0.41	-	-	-
O-Coumaric acid	0.73	-	-	-
p-Coumaric acid	0.34	-	-	-
Caffeic acid	0.34	-	-	-

This table was adapted from Marica *et al*., 2004

Solvent systems used in chromatographic system

Hexane extract = Hexane: dichloromethane, (50:50 v/v)

Dichloromethane extract = Dichloromethane: Hexane, (90:10 v/v)

Ethyl acetate extract = Ethyl acetate: dichloromethane, (90:10 v/v)

Ethanol extract = Ethanol: Hexane, (90:10)

Table 12: R_f Value of Flavonoids, Phenolic acids in the extracts of S*Toddalia asiatica*

Toddalia asiatica	R_f values			
TLC Systems	Hexane extracts	Dichloromethane extracts	Ethyl acetate extracts	Ethanol extracts
Flavone	-	-	-	0.88
3-Hydroxyflavone	-	-	0.83	-
Quercetin	-	-	0.22	-
Caffeic acid	-	-	0.22	-

This table was adapted from Marica *et al*., 2004

Solvent systems used in chromatographic system

Hexane extract = Hexane: dichloromethane, (50:50 v/v)

Dichloromethane extract = Dichloromethane: Hexane, (90:10 v/v)

Ethyl acetate extract = Ethyl acetate: dichloromethane, (90:10 v/v)

Ethanol extract = Ethanol: Hexane, (90:10)

Bidens Pilosa: Results obtained show that in comparison with standards R_f value in similar conditions, six spots in the chromatogram of hexane, two spot in the chromatogram of dichloromethane and ethyl acetate extract and two spots in the chromatogram of t ethanol extract of *Bidens pilosa* was equal to the standards (R_f values = 0.13, 0.20, 0.35, 0.42, 0.47, 0.5, 0.70, 0.75 and 0.85, respectively). These results indicated that *Bidens pilosa* may contain Morin, Apigenin, Quercetin, 7-Hydroxyflavone, 3,7-Dihydroxyflavone, Kaempferol, Ferulic acid, Flavanone, *O*-Coumaric acid, *p*-Coumaric acid, Flavone and Galangin (Table10). However, there are unknowns that need to be identified in further research.

Leonotis Nepetifolia: Results obtained show that in comparison with standards R_f value in similar conditions, four spots in the chromatogram of hexane, one spot in the chromatogram of dichloromethane, two spots in the chromatogram of ethyl acetate and t ethanol extract of *Leonotis nepetifolia* was equal to the standards (R_f values = 0.13, 0.20, 0.34, 0.41, 0.5, 0.72, 0.73, 0.87 and 0.88 respectively). These results indicated that *Leonotis nepetifolia* may contain Morin, Apigenin, 3, 6-Dihydroxyflavone, *p*-Coumaric acid, Caffeic acid, Kaempferol, 3, 7-Dihydroxyflavone, Galangin, Naringenin, 6-Hydroxyflavone, *O*-Coumaric acid and Flavone (Table 11). However, there are unknowns that need to be identified in further research.

Toddalia Asiatica: Results obtained show that in comparison with standards R_f value in similar conditions, three spots in the chromatogram of ethyl acetate extract, and t one spot in the chromatogram of t ethanol extract of *Toddalia asiatica* was equal to the standards (R_f values = 0.22, 0.83 and 0.88 respectively). These results indicated that S*Toddalia asiatica*n may contain Quercetin, 3-Hydroxyflavone, Caffeic acid and Flavone (Table 12). However, there are unknowns that need to be identified in further research.

Data collected: The retention factors, R_f of the hexane, dichloromethane, ethyl acetate and ethanol extracts of the eight herbs were determined and recorded.

Data Analysis: The calculated t value was obtained by comparing the sum of positive (+) and negative (-) results. The critical t value is obtained from significant test table using number of samples. Differences between the critical t-value and calculated t-values of the bioactive compounds of the herbal extracts were computed. For all the eight herbal species, the null hypothesis was retained because the calculated t-value was more than the critical t-value at $p \leq 0.05$.

CONCLUSION

Flavonoid and phenolic acid compounds are present in all the analysed selected eight medicinal herbs. The number of phytochemical compounds isolated by TLC and identified ranges from one to nine. The known flavonoid compounds in *Leonotis nepetifolia* are five*, Bidens pilosa* are four*,* Urtica *dioica, Senna didymobotrya and Toddalia asiatica* are three, *Warburgia ugandensis and Physalis peruviana* are two and none in *Carissa spinarum,* respectively. Known phenolic acid compounds in *Bidens pilosa* are nine*, Leonotis nepetifolia* are seven*, Senna didymobotrya* are six, *Physalis peruviana* are four*, Warburgia ugandensis* are three*, Toddalia asiatica* are two and one in *Carissa spinarum* and *Urtica dioica*. Further studies are needed with these herbs to identify the unknown flavonoids and Phenolic acids in the analysed herbal extracts, isolate, characterize and elucidate the structure of the bioactive compounds of the herbs which are responsible for the antimicrobial activity and other medicinal value.

ACKNOWLEDGMENT

The first author is greatly thankful to the Department of Chemistry and the Department of Food and Science Technology of Jomo Kenyatta University of Agriculture and Technology, for the provision of laboratory space for the extraction process and equipment to carry out this research and for use of the microbiology laboratory. My University Supervisors, Dr. E. Gatebe, Dr. L. Gitu and Dr. H. Rotich are gratefully acknowledged for the invaluable technical support provided to make this research a success. My sincere gratitude goes to all those that assisted me in one way or another during the course of the reported work.

REFERENCES

1. Kajaria, D.K., M. Gangwar, A.K. Sharma, G. Nath, Y.B. Tripathi, J.S. Tripathi and S.K. Tiwari, 2011. Comparative evaluation of phenol and flavonoid content of polyherbal drugs. Pharmacologyonline, 3: 1365-1373.
2. Gisesa, W.N.O., 2004. An Ethnopharmacological Investigation of Plants used by Abagusii Traditional Medical Practitioners, PhD Thesis, School of Pure and Applied Sciences, Kenyatta University.
3. Doss, A. and S.P. Anand, 2012. Preliminary Phytochemical Screening of Asteracantha longifolia and Pergularia daemia. World Applied Sciences Journal, 18(2): 233-235.
4. Kumar, A., R. Ilavarasan, T. Jayachandran, M. Decaraman, P. Aravindhan, N. Padmanaban and M.R.V. Krishna, 2009. Phytochemical investigation on tropical plants. Pakistan Journal of Nutrition, 8: 83-85.
5. Premkumar, G., R. Sankaranarayanan, S. Jeeva and K. Rajarathinam, 2011. Asian Pac. J. Trop. Biomed., 1: 169-172.
6. Nisha Raj, R.S. and P.M. Radhamany, 2010. Preliminary phytochemical and *in vitro* antioxidant properties of *Brunfelsia americana* L.R.S. Nisha Raj *et al.* / Journal of Pharmacy Research, 3(11): 2712-2713.
7. Kubmarawa, D., M.E. Khan, A.M. Punah and Hassan, 2008. Phytochemical screening and antibacterial activity of extracts from *Parkia clappertoniana* keay against human pathogenic bacteria. Journal of Medicinal Plant Research, 2(12): 352-355.

8. Savithramma, N., M. Linga Rao and D. Suhrulatha, 2011. Screening of medicinal plants for secondary metabolites. Middle-East Journal of Scientific Research, 8: 579-584.
9. Rupasinghe, H.P., C.J. Jackson, V. Poysa, C.D. Berado, J.D. Bewley and J. Jenkinson, 2003. Soyasapogenol A and B distribution in Soybean (Glycine max (L.) Merr.) In relation to seed physiology, genetic variability and growing location. Journal of Agricultural Food Chemistry, 51: 5888-5894.
10. Pawar, R., C. Gopalakrishnan and K.K. Bhutani, 2001. Dammarane triterpene saponin from Bacopa monniera as the superoxide inhibitor in polymophonuclear cells. Planta Med., 67: 752-754.
11. Marica, M.S., JIvona, S.B. Asja and M. Ana, 2004. Optimization of Chromatographic Conditions in Thin Layer Chromatography of Flavonoids and Phenolic Acids. Croatica Chemica Acta, 77: 361-366.
12. Shahidi, F. and M. Naczk, 2004. Phenolics in Food and Neutraceuticals: Sources, Applications and Health Effects, CRC Press, Boca Raton, FL.
13. Beckman, C.H., 2000. Resistance to *Fusarium wilt* in Banana: A Review. Physiology and Molecular Plant Pathology, 57: 101-110.
14. Wu, H., T. Haig, J. Prately, D. Lemerie and M. An, 2000. Journal of Agri. Food Chem., 48: 5321-5325.
15. Prior, R.L., H. Wu and L. Gu, 2006. Flavonoid metabolism and challenges to understanding mechanisms of health effects. J. Sci. Food Agric., 86: 2487-2491.
16. Kessler, M., G. Ubeaud and L. Jung, 2003. Anti- and pro-oxidant activity of rutin and quercetin derivatives. J. Pharm. Pharmacology, 55: 131-142.
17. Hassan, M.M., A.O. Oyewale, J.O. Amupitan, M.S. Abduallahi and E.M. Okonkwo, 2004. Preliminary Phytochemical and antibacterial investigation of crude extracts of the root bark of *Detarium microcarpum*, J. Chem. Soc. Igeria, 29: 26-29.
18. Anpin Raja, R.D., S. Jeeva, J.W. Prakash, M. Johnson and V. Irudayaraj, 2011. Antibacterial activity of selected ethnomedicinal plants from South India. Asian Pac. J. Trop. Med., 4: 375-378.
19. Mungole, A.J., R. Awati, A. Chaturvedi and P. Zanwar, 2010. Preliminary Phytochemical screening of Ipomoea obscura (L) -A hepatoprotective medicinal plant. International Journal of PharmTech Research CODEN (USA), 2(4): 2307-2312.
20. Narayana, K.R., M.S. Reddy, M.R. Chaluvadi and D.R. Krishna, 2001. Bioflavonoids classification, pharmacology, biochemical effects and therapeutic potential. Ind J. Pharmacol., 33: 2-16.
21. Shirwaikar, A., S. Malini and S.C. Kumari, 2003. Protective effect of *Pongamia pinnata* flowers against cisplatin and gentamicin induced nephrotoxicity in rats. Indian J. Exp. Biol., 1: 58-62.
22. Nisar, M., S. Ali and M. Qaisar, 2011. Preliminary Phytochemical Screening of Flowers, Leaves, Bark, Stem and Roots of *Rhododendron arboretum*. Middle-East Journal of Scientific Research, 10(4): 472-476.
23. Anpin Raja, R.D., J.W. Prakash and S. Jeeva, 2010. Antibacterial activity of some medicinal plants used by Kani tribe, southern Western Ghats, Tamilnadu, India. In: P.C. Trivedi, Editor. Ethnic Tribes and Medicinal Plants. Jaipur: Pointer Publishers, pp: 28-45.
24. Tirupathi Rao, G., K. Suresh Babu, J. Ujwal Kumar and P. Sujana, 2011. Veerabhadr Rao, Sreedhar AS. Anti-microbial principles of selected remedial plants from southern India. Asian Pac. J. Trop. Biomed., 1: 298-305.
25. Shalini, S. and P. Sampathkumar, 2012. Phytochemical screening and antimicrobial activity of plant extracts for disease management. International Journal Current Science, pp: 209-218.
26. Panyaphu, K., P Sirisa-ard, P.N. Na Ubol, S. Nathakarnkitku, S. Chansakaow and T. Van On, 2012. Phytochemical, antioxidant and antibacterial activities of medicinal Plants used in Northern Thailand as postpartum herbal bath recipes by the Mien (Yao) community. Phytopharmacology, 2(1): 92-105.
27. Balakumar, S., S. Rajan, T. Thirunalasundari and S. Jeeva, 2011. Antifungal activity of *Aegle marmelos* (L.) Correa (Rutaceae) leaf extract on dermatophytes. Asian Pac. Jo. Trop. Biomed., 1: 309-312.