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A PRELIMINARY PHYTOCHEMICAL SCREENING OF MEDICINAL PLANTS: A CASE STUDY OF SELECTED PLANT SPECIES AT THREE PHENOLOGICAL STAGES

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ABSTRACT

Qualitative phytochemical determination of eight selected ethnomedicinally important plant species was carried out on crude ethanolic extract, n-Hexane, chloroform and ethyl-acetate fractions. These plant species were collected at three phenological stages from Tall Dardyal Hills, Tehsil Kabal, District Swat which were used by the locals in traditional indigenous health care system. These plant species were analysed for the presence and absence of twelve important secondary metabolites such as alkaloids, anthocyanin and betacyanins, anthraquinones, cardiac glycosides, flavonoids, glycosides, amino acids and proteins, reducing sugars, saponins, steroids, tannins and terpenoids. The current study revealed that secondary metabolites were predominantly found in ethanolic crude extract followed by ethyl-acetate and chloroform fractions and rarely observed in n-hexane fraction. The investigation is of particular significance to know the presence of these phytochemicals in various fractions and to provide a base line data for its further quantification and utilization in pharmaceutical industry.

Key words: Ethanolic crude extract, medicinal plants, phenological stage, Phytochemical screening.

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INTRODUCTION

Nature is the prime source of high phytochemical diversity. Many of them possess potential discrete bioactivities beyond those related to minerals and vitamins. They could provide health benefits in various

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forms as substrates for biochemical processes, cofactors and inhibitors of enzymatic reactions, squestrants of undesirable substances in intestines, scavengers of toxic chemicals, selective inhibitors of harmful intestinal bacteria, enhancers for absorption of essential nutrients etc. Such phytochemicals include alkaloids, terpenoids, phenolics and fibres (Dillard and German, 2000; Webb, 2013). More than 4,000, phytochemicals have been recorded so far. Out of these, 150 phytochemicals have been investigated in detail (American Cancer Society, 2002). These have been classified on the bases of their physical and chemical properties and protective nature (Mathai, 2000). Phytochemicals are responsible for the color, odor and flavor of plant foods. Phytochemicals, also grouped as phytonutrients, are found in vegetables, herbs, whole grains, fruits, seeds, nuts, legumes and spices (Webb, 2013). Phytochemicals rich diet is important for human health due to their considerable antioxidant properties (Dixon *et al*., 2005; Crozier *et al*., 2009). Therefore the use of of different plants for their potential uses need to be explored in developing countire (Shah et al. 2014; Shad et al. 2016).

MATERIALS AND METHODS

Qualitative chemical analysis of eight selected medicinal plants was carried out on crude ethanolic extract, *n*-Hexane, chloroform and ethyl-acetate fractions. They were collected at three phenological stages from Tall Dardyal Hills, Tehsil Kabal, District Swat, Pakistan. Collection at different plant growth stages was aimed to know which stage possess secondary metabolites. These plant species were selected on the basis of their traditional indigenous medicinal value. Plant materials were shade dried at room temperature for 20 days. Then crushed to fine powder on grinding machine and preserved in plastic bags for chemical evaluation.

List of medicinal plant species selected for phytochemical screening at three phenological stages.

S. No.	Plant Species	Families
1.	*Dysphania botrys* L.	Amaranthaceae
2.	*Sarcococa saligna* (D.Don) Muell. Arg.	Buxaceace
3.	*Isodon rugosus* (Wall. ex Benth.) Codd	Lamiaceae
4.	*Origanum vulgare* L.	Lamiaceae
5.	*Salvia canariensis* L.	Lamiaceae
6.	*Thymus linearis* Benth.	Lamiaceae
7.	*Daphne mucronata* Royle.	Thymelaeaceae
8.	*Wikstroemia canescens* Wall.ex Meisn.	Thymelaeaceae

Phytochemical Screening

Qualitative analysis of secondary metabolites was carried out on crude ethanolic extract, n-hexane, chloroform and ethyl acetate fractions following the procedures (Uddin *et al*., 2011a; 2012b) with some modification to know which extract/fraction show the presence of metabolites. The crude extracts obtained were analysed for different secondary metabolites as follows:

Test for alkaloids: 0.2g of each crude extract was warmed after adding 2ml of H_2SO_4 (2%) for two minutes. The mixture was filtered. Few drops of Dragendrof's reagents were added to the filtrate. The appearance of orange red precipitate indicated the presence of alkaloids.

Test for tannins: 0.2g of each extract was taken in a test tube. Distilled water was added to it. Warmed the mixture on water bath and filtered it. Few drops of Ferric chloride ($FeCl_3$) were added to the filtrate. Dark green colour solution indicated the presence of tannins.

Test for anthraquinones: 0.5g of each extract was taken in a test tube. Add few drops of 10 % HCl to it. The reaction mixture was filtered and cooled it at room temperature. Equal volume of Chloroform ($CHCL_3$) was mixed with each filtrate. The filtrate was heated after adding few drops of 10% NH_3. The presence of anthraquinones was confirmed by obtaining rose-pink coloration.

Test for glycosides: 0.6g of each extract was added few drops of concentrated solution of HCl, NaOH. It was followed by few drops of Fehling's solution. The reaction mixture was heated. Formation of red ppt indicated the presence of glycosides.

Test for reducing sugars: Distilled water was added to 0.5g of each extract. It was shaken and filtered. Mixed few drops of Fehling's solution with the filtrate and boiled the reaction mixture for few minutes. Formation of an orange red ppt indicated the presence of reducing sugars.

Test for saponins: 5.0ml distilled water was added to 0.2g of each extract, vigorously shaked it. The appearance of creamy small bubbles indicated the presence of saponins.

Test for flavonoids: 10% NaOH was mixed with 0.2g of each extract and then added few drops of concentrated HCl. The presence of flavonoids was confirmed by changing the yellow colour solution into colourless solution.

Test for steroids: 0.5g of each extract was mixed with 2.0ml of acetic anhydride and H_2SO_4 each. The appearance of violet blue or green colour indicated the presence of steroids.

Test for terpenoids: Chloroform was added to 0.2g of each extract. 3.0ml of concentrated H_2SO_4 was carefully mixed to make a layer. At

the interface a reddish brown coloration was observed which showed terpenoids positive results.

Test for betacyanins and anthocyanin: 1.0ml of NaOH (2N) was added to 2.0g of each extract and heated for 5 minutes at 100°C. Bluish green colour appearance indicated anthocyanin positive results.

Test for amino acids and proteins: Few drops of 0.2% Ninhydrin was added to 2.0g of each extract and heated on spirit lamp for 5 minutes. The blue colour appearance indicated positive results for amino acids and proteins.

Test for cardiac glycosides: 1.0ml of glacial acetic acid was mixed with 2.0g of each extract. The mixture was added 5.0% $FeCl_3$ and few drops of concentrated H_2SO_4. The presence of cardiac glycosides was confirmed by the appearance of greenish blue colour.

RESULTS AND DISCUSSION

Qualitative analysis of twelve important secondary metabolites in selected ethnomedicinal plants is given below,

Alkaloids

Alkaloids contents were found in ethanolic crude extract, chloroform and ethyl-acetate fractions but were absent in n-hexane fraction of *Salvia canariensis* at three phenological stages. Alkaloids contents were present in ethanolic crude extract at three phenological stages of *Wikstroemia canescence* and also in ethyl-acetate fraction at post-reproductive stage. Chloroform and n-hexane fraction showed negative results at three phenological stages. At three phenological stages *Sarcococca saligna*, *Daphne mucronata* and *Thymus linearis* contained alkaloids in all fractions except for n-hexane fraction. Ethanolic crude extract of *Dysphania botrys* has alkaloid contents at three phenological stages. It was also present in ethyl-acetate fraction at pre and post-reproductive stages. *Isodon rugosus* contained alkaloids at various phenological stages in ethanolic crude extract and in chloroform and ethyl-acetate fractions at pre-reproductive stages. N-hexane fraction showed negative result. Crude ethanolic extraction and ethyl-acetate fraction of *Origanum vulgare* have alkaloid contents at various phenological stages and chloroform fraction at post-reproductive stage. It was found absent in n-hexane fraction at all phenological stages (Table-1 to Table-8).

Anthocyanin and Betacyanins

Salvia canariensis showed the presence of anthocyanin and betacyanins in all fractions at post-reproductive stage. *Wikstroemia canescence* showed positive results in ethanolic crude extract at three phenological stages. Anthocyanin and betacyanins was found in ethyl-acetate fraction at pre-reproductive and reproductive stages. Anthocyanin and betacyanins existed at pre-reproductive stage in n-

hexane and at reproductive stage in chloroform fractions. In *Sarcococca saligna* anthocyanin and betacyanins were present in all fractions except n-hexane fraction at post-reproductive stage. *Dysphania botrys, Origanum vulgare, Thymus linearis* and *Daphne mucronata* showed negative results for anthocyanin and betacyanins in all fractions at three phenological stages. In *Isodon rugosus* anthocyanin and betacyanins were present in crude ethanolic extract, chloroform and ethyl-acetate fractions at pre-reproductive stage. They were also found in crude ethanolic extract at reproductive stage. Post-reproductive stage showed their absence in all fractions (Table-1 to Table-8).

Anthraquinones

Anthraquinones were not available in all fractions at three phenological stages of *Wikstroemia canescence*, *Sarcococca saligna* and *Daphne mucronata.* In *Dysphania botrys* and *Origanum vulgare* showed the presence of anthraquinones in crude ethanolic extract at pre-reproductive, reproductive and post-reproductive stages. It was also observed in ethyl-acetate fraction at pre-reproductive and post-reproductive stages and in chloroform fraction at pre-reproductive stage. The absence of anthraquinones was recorded in n-hexane fraction at three phenological stages. In *Salvia canariensis* absence of anthraquinones was observed in all fractions at pre-reproductive and post-reproductive stages. It was found only in crude ethanolic and chloroform fractions at reproductive stage. *Thymus linearis* showed the presence of anthraquinones in ethanolic crude extract at three phenological stages. It was also observed in ethyl-acetate fraction at pre-reproductive and post-reproductive stages. At reproductive stage, anthraquinones were also present in chloroform fraction. At various phenological stages, anthraquinones were absent in n-hexane fraction. In *Isodon rugosus,* anthraquinones were absent in all fractions at reproductive and post-reproductive stages but were found only in ethanolic crude extract at pre-reproductive stage (Table-1 to Table-8).

Cardiac glycosides

Dysphania botrys has cardiac glycosides in ethanolic fraction at three phenological stages, also in ethyl-acetate fraction at pre-reproductive stage. Chloroform and n-hexane fractions showed negative results for cardiac glycosides. In *Origanum vulgare,* cardiac glycosides were only observed in ethanolic crude extract at three phenological stages. At reproductive stage of *Salvia canariensis*, all fractions showed the presence of cardiac glycosides. In ethanolic crude extract and n-hexane fraction, cardiac glycosides were observed at pre and post-reproductive stages. Ethyl-acetate at post-reproductive stage also showed positive results. In chloroform and ethyl-acetate fractions at pre-reproductive stage and chloroform faction at post-reproductive

stage absence of cardiac glycosides were noted. *Thymus linearis* showed the presence of cardiac glycosides in all fractions at three phenological stages, except n-hexane fraction at pre and post-reproductive stages. In *Daphne mucronata*, cardiac glycosides were present in all fractions at three phenological stages except in n-hexane, chloroform and ethyl-acetate fractions at post-reproductive stage. Ethanolic crude extract of *Sarcococca saligna* showed presence of cardiac glycosides at three phenological stages and also in n-hexane fraction at pre-reproductive stages. The rest of the fractions showed their absence. In *Isodon rugosus,* ethanolic crude extract indicated the presence of cardiac glycosides at three phenological stages. Ethyl-acetate fraction at pre-reproductive and reproductive stages also showed its presence. It was found absent in n-hexane and chloroform fractions at pre-reproductive and reproductive and post-reproductive stages. *Wikstroemia canescence* showed the presence of cardiac glycosides in all fractions at three phenological stages except in n-hexane and chloroform fractions at post-reproductive stage (Table-1 to Table-8).

Flavonoids

At post-reproductive stage of *Dysphania botrys,* n-hexane and chloroform fractions showed negative results for flavonoids. All other fractions showed presence of flavonoids at pre-reproductive and reproductive stages. All fractions showed presence of flavonoids at three phenological stages of *Origanum vulgare* and *Wikstroemia canescence*. At post-reproductive stage, flavonoids was absent in n-hexane fraction. Flavonoids were confirmed in all fractions at three phenological stages of *Salvia canariensis* and *Daphne mucronata*. Chloroform fraction at post-reproductive stage and n-hexane fraction at pre-reproductive stage of *Thymus linearis* showed negative results for flavonoids. All other fractions at three phenological stages indicated the presence of flavonoids. At pre-reproductive of *Sarcococa saligna*, flavonoids were present in all fractions. At reproductive stage, it was available in all fractions except n-hexane fraction. At post-reproductive, flavonoids were present in ethanolic crude extract and ethyl-acetate fraction. Chloroform and n-hexane fractions showed the absence of flavonoids. *Isodon rugosus* indicated the presence of flavonoids in all fractions at pre-reproductive stage. At reproductive and post-reproductive stages, flavonoids were present in all fractions except n-hexane fraction (Table-1 to Table-8).

Glycosides

In *Dysphania botrys,* glycosides were present in ethanolic crude extract and ethyl-acetate fraction at pre-reproductive stage. Ethanolic crude extract showed the presence of glycosides at reproductive and post-reproductive stages. The rest of the fractions showed negative

results for glycosides. At pre-reproductive and reproductive stages of *Origanum vulgare,* only n-hexane fraction showed the absence of glycosides. At post-reproductive stage, glycosides were present in ethanolic crude extract only. Other fractions showed its absence. In *Salvia canariensis,* glycosides were present only in ethanolic crude extract at reproductive stage and were found absent in rest of the fractions and growth stages. At pre-reproductive stage of *Thymus linearis*, ethanolic crude extract and chloroform fractions showed positive results for glycosides. It was also found absent in the rest of the fractions. At reproductive stage, glycosides were absent in chloroform and ethyl-acetate fractions and were present in other two fractions. At post-reproductive stage, ethanolic crude extract showed the presence of glycosides and its absence in other three fractions. Glycosides were present only in ethanolic crude extract at reproductive stage of *Daphne mucronata* and *Wikstroemia canescence.* The rest of the fractions at various phenological stages showed negative results for glycosides. At post-reproductive stage of *Sarcococca saligna,* ethanolic crude extract and ethyl-acetate fractions showed positive results for glycosides. The rest of the fractions showed their absence at various phenological stages. At pre-reproductive stage of *Isodon rugosus*, ethanolic crude extract contained glycosides. At reproductive stage, ethanolic crude extract and ethyl-acetate indicated the presence of glycosides and the rest of the fractions showed its absence. At post-reproductive stage, glycosides were only found in ethanolic crude extract (Table-1 to Table-8).

Amino acids and Proteins

At pre-reproductive stage of *Dysphania botrys,* amino acids and proteins were found absent in n-hexane fraction only. At reproductive stage, they were present in ethanolic crude extract. At post-reproductive stage, the presence of amino acids and proteins were noted in ethanolic crude extract and ethyl-acetate fractions. The rest of the fractions showed their absence. *Origanum vulgare* showed positive results for amino acids and proteins in ethanolic crude extract at reproductive and post-reproductive stages. They were found absent in the rest of the fractions at various phenological stages. Amino acid and proteins were absent in chloroform fraction at post-reproductive stage of *Salvia canariensis* and in n-hexane fraction of *Thymus linearis*. Positive results were shown by all fractions at various phenological stages. In *Daphne mucronata*, amino acids and proteins were absent in two fractions i.e. n-hexane and chloroform fraction at reproductive and post-reproductive stages respectively. They were found in the rest of the fractions at various phenological stages. *Sarcococca saligna* showed the presence of amino acids and proteins only in ethanolic crude extract at post-reproductive stage. It was found

absent in the rest of the fractions at various phenological stages. At pre-reproductive and reproductive stages of *Isodon rugosus*, only n-hexane fraction showed absence of amino acids and proteins. At post-reproductive stage, they were found only in ethanolic crude extract. In *Wikstroemia canescence*, amino acids and proteins showed positive result in ethanolic crude extract at three phenological stages. Amino acids and proteins were also present in chloroform and ethyl-acetate fractions at pre-reproductive and reproductive stages. The rest of fractions showed negative results (Table-1 to Table-8).

Reducing sugars

At pre-reproductive stage of *Dysphania botrys* reducing sugars were absent only in n-hexane fraction. It was found in ethanolic crude extract at reproductive and post-reproductive stages. The rest of the fractions showed its absence. *Origanum vulgare* showed similarity with *Dysphania botrys* at pre-reproductive stage regarding the presence and absence of reducing sugars. At reproductive and post-reproductive stages, reducing sugars were found in ethanol crude extract and ethyl-acetate fractions. Ethanolic crude extract of *Salvia canariensis* at pre-reproductive and reproductive stages showed positive results for reducing sugars. At post-reproductive stage, reducing sugars were found in all fractions. In *Thymus linearis*, ethanolic crude extract and ethyl-acetate fraction indicated the presence of reducing sugars at three phenological stages. At post-reproductive stage, chloroform fraction also showed the presence of reducing sugars. At pre-reproductive stage of *Sarcococca saligna,* all fractions showed the absence of reducing sugars. At reproductive and post-reproductive stages, reducing sugars were present only in ethanolic crude extract. Ethanolic crude extract and n-hexane fraction indicated the presence of reducing sugars at pre and post-reproductive stages of *Daphne mucronata.* At reproductive stage, reducing sugars were only found in ethanolic crude extract. The rest of the fractions showed negative results for reducing sugars at various phenological stages. Reducing sugars were found in ethanolic crude extract at three phenological stages of *Isodon rugosus.* Chloroform fraction at pre-reproductive and ethyl-acetate extract at pre and post-reproductive stages also indicated the presence of glycosides. *Wikstroemia canescence* contained reducing sugars only in ethanolic crude extract at three phenological stages. It was found absent in the rest of the fractions (Table-1 to Table-8).

Saponins

Saponins were present in ethanolic crude extract at three phenological stages of *Dysphania botrys.* Saponins were also observed in ethyl-acetate fractions at pre-reproductive and post-reproductive stages. At three phenological stages of *Origanum vulgare*, n-hexane

fraction showed the absence of saponins. Saponins absence was also observed in chloroform fraction at pre-reproductive stage. Saponins were found in all fractions at pre-reproductive stage of *Salvia canariensis*. At reproductive and post-reproductive stages, ethanolic crude extract and ethyl-acetate fraction showed positive results for saponins. The rest of the fractions showed the absence of saponins. In ethanolic crude extract and ethyl-acetate fraction of *Thymus linearis* showed the presence of saponins at pre and post-reproductive stages. At reproductive stage saponins were absent only in n-hexane fraction. In *Daphne mucronata,* saponins were found in ethanolic crude extract at three phenological stages and only in ethyl-acetate fraction at pre-reproductive stage. The rest of the fractions showed the absence of saponins. *Sarcococca saligna* and *Isodon rugosus* showed similarity with *Thymus linearis* in the presence of saponins at pre-reproductive and reproductive stages. *S. saligna* showed similarity at post-reproductive with *D. mucronata* regarding the presence of saponins. In *I. rugosus*, saponins were only absent in n-hexane fraction at post-reproductive stage. Ethanolic crude extract and ethyl-acetate fraction of *Wikstroemia canescence* showed positive results for saponins at pre-reproductive and reproductive stages. At post-reproductive stage, only ethanolic crude extract showed the presence of saponins. The rest of the fractions showed negative results at various phenological stages (Table-1 to Table-8).

Steroids

In *Dysphania botrys,* at three phenological stages, steroids were only absent in ethyl-acetate fraction and were present in the rest of fractions. Steroids were available in all fractions at pre-reproductive stage of *Origanum vulgare.* At reproductive stage, saponins were only present in ethanolic crude extract. At post-reproductive stage, only ethyl-acetate fraction showed negative results for steroids. In *Salvia canariensis* steroids were observed in all fractions at three phenological stages. At pre-reproductive stage of *Thymus linearis*, steroids were unavailable in all fractions. At reproductive stage, all fractions showed positive results for steroids. At post-reproductive stage, steroids were only absent in n-hexane fraction. All fractions showed positive results for steroids at reproductive and post-reproductive stages of *Daphne mucronata.* Only ethyl-acetate fraction at pre-reproductive stage showed negative result for steroids. In *Sarcococca saligna,* steroids were found in all fractions at three phenological stages except ethyl-acetate fraction at reproductive and post-reproductive stages. Steroids were only present in ethanolic crude extract of *Isodon rugosus* at post-reproductive stage. *Wikstroemia canescence* showed the absence of steroids only in ethyl-

acetate fraction at reproductive and post-reproductive stages and presence in rest of the fractions (Table-1 to Table-8).

Tannins

In *Dysphania botrys,* the presence of tannins was observed in ethanolic crude extract and ethyl-acetate fraction at pre and post-reproductive stages. At reproductive stage, tannins were confirmed only in ethanolic crude extract. *Origanum vulgare* showed similarity at three phenological stages with pre and post-reproductive stages of *D. botrys* regarding the presence of tannins. At pre-reproductive stage of *Salvia canariensis,* tannins were present in ethanolic crude and ethyl-acetate fraction. At reproductive stage, tannins were absent only in n-hexane fraction and were present in all fractions at post-reproduction stage. Ethanolic crude extract and ethyl-acetate fraction of *Thymus linearis* showed the presence of tannins at three phenological stages. It was absent in the rest of the fractions. The presence of tannins was noted in ethanolic crude extract at three phenological stages of *Daphne mucronata.* It was also found in n-hexane fraction and chloroform fractions at pre and post-reproductive stages of *D. mucronata.* The absence of tannins was observed in the rest of the fractions. Ethanolic crude extract and ethyl-acetate fractions showed the presence of tannins at three phenological stages of *Sarcococca saligna* and *Isodon rugosus.* Tannins were absent in the rest of the fractions. Tannins were found only in ethanolic extract of *Wikstroemia canescence* at three phenological stages (Table-1 to Table-8).

Terpenoids

Terpenoids were observed in all fractions at three phenological stages of *Thymus linearis* except ethyl-acetate fraction at pre-reproductive stage. *Origanum vulgare* showed the presence of terpenoids in all fractions at various phenological stages but were found absent in ethyl-acetate fraction at three phenological stages. In *Dysphania botrys,* all fractions have terpenoids at pre-reproductive stage. At reproductive and post-reproductive stages, terpenoids were observed in all fractions except ethyl-acetate fraction. *Salvia canariensis* showed the presence of terpenoids in all fractions at three phenological stages. Positive results for terpenoids were shown by all fractions except ethyl-acetate fraction at three phenological stages of *Isodon rugosus* and *Wikstroemia canescence*. *Daphne mucronata* showed negative results for terpenoids in ethyl-acetate fraction at reproductive and post-reproductive stages. The presence of terpenoids was observed in all fractions at pre-reproductive stages. Ethanolic crude extract, chloroform and n-hexane fractions at reproductive and post-reproductive stages also showed positive results. The presence of terpenoids was observed in all fractions at pre- reproductive stage of *Sarcococca saligna*. Terpenoids were found at reproductive stage in

ethanolic crude extract and ethyl-acetate fraction and in all fractions at post-reproductive stage except ethyl-acetate fraction (Tables-1 to 8).

Investigation on twelve phytochemicals in the selected plants revealed that most of them were found in ethanolic crude extract as compared to other fractions. The reason for the presence and absence of secondary metabolites mainly due to polar and non-polar nature of both the extracts and secondary metabolites. The order of polarity from high to low is ethanol crude extract>chloroform fraction>ethyl-acetate fraction>n-hexane fraction. Steroids and terpenoids are non-polar. Saponins and tannins are double polar as compared to other secondary metabolites. The current study confirmed the evidence that ethanolic solvent is more appropriate than other solvents in extracting secondary metabolites of plants (Ahmad *et al*., 1998; Cowan, 1999; Emad *et al*., 2009). Water-alcohol extraction method is used for extraction of phenolic compounds and its presence can also be observed separately in both extracts (Perry *et al*., 2001). Terpenes constitute an important class of phytonutrients in grains, green foods and soy plants (Harborne and Baxter, 1993). Phytochemical-rich foods decrease the risk of diabetes, improving insulin sensitivity thereby indirectly prevents weight gain. Most probably, also reduces inflammation (Caster *et al*., 2010; Castejon and Casado, 2011). Phytochemicals rich diet is important for human health due to their considerable antioxidant properties (Dixon *et al*., 2005; Crozier *et al*., 2009). Flavonoids, phenolic acids and tannins are the major groups which contain these antioxidant compounds. Of these, about 19% antioxidant capacity of the total diet is accounted for by tannins (Floegel *et al*., 2010).

Terpenoids exhibits diverse functions from deadly toxic to entirely edible. It also functions as antimicrobial and ecologically symbiotes attractants for pollination, seed dispersal and as anti-germinative phytotoxic allelopaths. It confers fragrance, flavor, and taste to plant products (De Almeida, 2010; De Martino, 2010). From ecological point of view, alkaloids, by and large, act as toxins and feeding deterrent to herbivores and insects (Harborne, 1993). Natural products play a key role in the prevention and treatment of various human diseases. Among them, terpenoids is the most widespread and largest group of secondary metabolites. Therapeutic uses of some terpenoids have been recognized for centuries as anti-inflammatory, antimicrobial, antifungal, antiviral, antiparasitic, antihyperglycemic and antitumoral agents (Paduch *et al*., 2007; Heras and Hortelano, 2009). Flavonoids are the most varied group of phytochemicals. More than 6,000 flavonoids have been described in plant foods (Arts and Hollman, 2005). Flavonoids are an essential component of animal and human diet. Generally, flavonoids are responsible for taste, colour,

prevention of fats oxidation in food and it also provides protection to enzymes and vitamins (Yao *et al*., 2004).

Besides imparting attractive colours to food products, some red pigments of anthocyanins and betacyanins activate free radical scavenging and some related antioxidant activities (Cai *et al*., 2003; Stintzing *et al*., 2005; Tesoriere *et al*., 2008; Fracassetti *et al*., 2013; Li *et al*., 2013). Anthocyanins and betalains, used as food colorants, may confer protection against some oxidative stress-related infirmities in humans (Lee *et al*., 2005; Allegra *et al*., 2007; Wallace, 2011; Christensen *et al*., 2012; Hobbs *et al*., 2012). Saponins modulate cell mediated immunity, enhance antibody production (Oda *et al*., 2000), monocytes production (Delmas *et al*., 2000; Yui *et al*., 2001) as well as show inflammation (De Oliveira *et al*., 2001; Haridas *et al*., 2001).

CONCLUSION

In plant extraction process, the use of alcohols maximizes the bioavailability of active constituents. This study provides a baseline for further scientific exploration, especially in antimicrobial activity.

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Table-1. Phytochemical screening of ethanolic (Crude extract), n-hexane, chloroform and ethyl-acetate fractions of *Dysphania botrys* L.

Phytochemical constituents	Pre-reproductive stage				Reproductive stage				Post-reproductive stage			
	1	2	3	4	1	2	3	4	1	2	3	4
Alkaloids	+	–	–	+	+	–	–	–	+	–	–	+
Anthocyanin and Betacyanins	–	–	–	–	–	–	–	–	–	–	–	–
Anthraquinones	+	–	+	+	+	–	–	–	+	–	–	+
Cardiac glycosides	+	–	–	+	+	–	–	–	+	–	–	–
Flavonoids	+	+	+	+	+	+	+	+	+	–	–	+
Glycosides	+	–	–	+	+	–	–	–	+	–	–	–
Amino acids and Proteins	+	–	+	+	+	–	–	–	+	–	–	+
Reducing sugars	+	–	+	+	+	–	–	–	+	–	–	–
Saponins	+	–	–	+	+	–	–	–	+	–	–	+
Steroids	+	+	+	–	+	+	+	–	+	+	+	–
Tannins	+	–	–	+	+	–	–	–	+	–	–	+
Terpenoids	+	+	+	+	+	+	+	–	+	+	+	–

Keys: + Presence, - Absence, 1. Ethanolic crude extract 2. n-hexane fraction 3. Chloroform fraction 4. Ethyl-acetate fraction

Table-2. Phytochemical screening of ethanolic (Crude extract), n-hexane, chloroform and ethyl-acetate fractions of *Isodon rugosus* (Wall.ex Benth.) Codd

Phytochemical constituents	Pre-reproductive stage				Reproductive stage				Post-reproductive stage			
	1	2	3	4	1	2	3	4	1	2	3	4
Alkaloids	+	–	+	+	+	–	–	–	+	–	–	–
Anthocyanin and Betacyanins	+	–	+	+	+	–	–	–	–	–	–	–
Anthraquinones	+	–	–	–	–	–	–	–	–	–	–	–
Cardiac glycosides	+	–	–	+	+	–	–	+	+	–	–	–
Flavonoids	+	+	+	+	+	–	+	+	+	–	+	+
Glycosides	+	–	–	–	+	–	–	+	+	–	–	–
Amino acids and Proteins	+	–	+	+	+	–	+	+	+	–	–	–
Reducing sugars	+	–	+	+	+	–	–	–	+	–	–	+
Saponins	+	–	–	+	+	–	–	+	+	–	+	+
Steroids	–	–	–	–	–	–	–	–	+	–	–	–
Tannins	+	–	–	+	+	–	–	+	+	–	–	+
Terpenoids	+	+	+	–	+	+	+	–	+	+	+	–

Keys: + Presence, - Absence 1. Ethanolic crude extract 2. n-hexane fraction 3. Chloroform fraction 4. Ethyl-acetate fraction

Table-3. Phytochemical screening of ethanolic (Crude extract), n-hexane, chloroform and ethyl-acetate fractions of *Origanum vulgare* L.

Phytochemical constituents	Pre-reproductive stage				Reproductive stage				Post-reproductive stage			
	1	2	3	4	1	2	3	4	1	2	3	4
Alkaloids	+	–	–	+	+	–	–	+	+	–	+	+
Anthocyanin and Betacyanins	–	–	–	–	–	–	–	–	–	–	–	–
Anthraquinones	+	–	+	+	+	–	–	–	+	–	–	+
Cardiac glycosides	+	–	–	–	+	–	–	–	+	–	–	–
Flavonoids	+	+	+	+	+	+	+	+	+	–	+	+
Glycosides	+	–	+	+	+	–	+	+	+	–	–	–
Amino acids and Proteins	–	–	–	–	+	–	–	–	+	–	–	–
Reducing sugars	+	–	+	+	+	–	–	+	+	–	–	+
Saponins	+	–	–	+	+	–	+	+	+	–	+	+
Steroids	+	+	+	+	+	–	–	–	+	+	+	–
Tannins	+	–	–	+	+	–	–	+	+	–	–	+
Terpenoids	+	+	+	–	+	+	+	–	+	+	+	–

Key: + Presence, - Absence, 1. Ethanolic crude extract 2. n-hexane fraction 3. Chloroform fraction 4. Ethyl-acetate fraction

Table-4. Phytochemical screening of ethanolic (Crude extract), n-hexane, chloroform and ethyl-acetate fractions of *Thymus linearis* Benth.

Phytochemical constituents	Pre-reproductive stage				Reproductive stage				Post-reproductive stage			
	1	2	3	4	1	2	3	4	1	2	3	4
Alkaloids	+	–	+	+	+	–	+	+	+	–	+	+
Anthocyanin and Betacyanins	–	–	–	–	–	–	–	–	–	–	–	–
Anthraquinones	+	–	–	+	+	–	+	–	+	–	+	+
Cardiac glycosides	+	–	+	+	+	+	+	+	+	–	+	+
Flavonoids	+	–	+	+	+	+	+	+	+	+	–	+
Glycosides	+	–	+	–	+	+	–	–	+	–	–	–
Amino acids and Proteins	+	+	+	+	+	+	+	+	+	–	+	+
Reducing sugars	+	–	–	+	+	–	–	+	+	–	+	+
Saponins	+	–	–	+	+	–	+	+	+	–	–	+
Steroids	–	–	–	–	+	+	+	+	+	–	+	+
Tannins	+	–	–	+	+	–	–	+	+	–	–	+
Terpenoids	+	+	+	–	+	+	+	+	+	+	+	+

Key: + Presence, - Absence, 1. Ethanolic crude extract 2. n-hexane fraction 3. Chloroform fraction 4. Ethyl-acetate fraction

Table-5. Phytochemical screening of ethanolic (Crude extract), n-hexane, chloroform and ethyl-acetate fractions of *Salvia canariensis* L.

Phytochemical constituents	Pre-reproductive stage				Reproductive stage				Post-reproductive stage			
	1	2	3	4	1	2	3	4	1	2	3	4
Alkaloids	+	–	+	+	+	–	+	+	+	–	+	+
Anthocyanin and Betacyanins	–	–	–	–	–	–	–	–	+	+	+	+
Anthraquinones	–	–	–	–	+	–	+	–	–	–	–	–
Cardiac glycosides	+	+	–	–	+	+	+	+	+	+	–	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	–	–	–	–	+	–	–	–	–	–	–	–
Amino acids and Proteins	+	+	+	+	+	+	+	+	+	+	–	+
Reducing sugars	+	–	–	–	+	–	–	–	+	+	+	+
Saponins	+	+	+	+	+	–	–	+	+	–	–	+
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	–	–	+	+	–	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+

Key: + Presence, - Absence, 1. Ethanolic crude extract 2. n-hexane fraction 3. Chloroform fraction 4. Ethyl-acetate fraction

Table-6. Phytochemical screening of ethanolic (Crude extract), n-hexane, chloroform and ethyl-acetate fractions of *Wikstroemia canescence* Wall. ex Meisn.

Phytochemical constituents	Pre-reproductive stage				Reproductive stage				Post-reproductive stage			
	1	2	3	4	1	2	3	4	1	2	3	4
Alkaloids	+	–	–	–	+	–	–	–	+	–	–	+
Anthocyanin and Betacyanins	+	+	–	+	+	–	+	+	+	–	–	–
Anthraquinones	–	–	–	–	–	–	–	–	–	–	–	–
Cardiac glycosides	+	+	+	+	+	+	+	+	+	–	–	+
Flavonoids	+	+	+	+	+	+	+	+	+	–	+	+
Glycosides	–	–	–	–	+	–	–	–	–	–	–	–
Amino acids and Proteins	+	–	+	–	+	–	–	+	+	–	–	–
Reducing sugars	+	–	–	–	+	–	–	–	+	–	–	–
Saponins	+	–	–	+	+	–	–	+	+	–	–	–
Steroids	+	+	+	+	+	+	+	–	+	+	+	–
Tannins	+	–	–	–	+	–	–	–	+	–	–	–
Terpenoids	+	+	+	–	+	+	+	–	+	+	+	–

Key: + Presence, - Absence, 1. Ethanolic crude extract 2. n-hexane fraction 3. Chloroform fraction 4. Ethyl-acetate fraction

Table-7. Phytochemical screening of ethanolic (Crude extract), n-hexane, chloroform and ethyl-acetate fractions of *Daphne mucronata* Royle.

Phytochemical constituents	Pre-reproductive stage				Reproductive stage				Post-reproductive stage			
	1	2	3	4	1	2	3	4	1	2	3	4
Alkaloids	+	–	+	+	+	–	+	+	+	–	+	+
Anthocyanin and Betacyanins	–	–	–	–	–	–	–	–	–	–	–	–
Anthraquinones	–	–	–	–	–	–	–	–	–	–	–	–
Cardiac glycosides	+	+	+	+	+	+	+	+	+	–	–	–
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	–	–	–	–	+	–	–	–	–	–	–	–
Amino acids and Proteins	+	+	+	+	+	–	+	+	+	+	–	+
Reducing sugars	+	+	–	–	+	–	–	–	+	+	–	–
Saponins	+	–	–	+	+	–	–	–	+	–	–	–
Steroids	+	+	+	–	+	+	+	+	+	+	+	+
Tannins	+	+	–	–	+	–	–	–	+	–	+	–
Terpenoids	+	+	+	+	+	+	+	–	+	+	+	–

Key: + Presence, - Absence, 1. Ethanolic crude extract 2. n-hexane fraction 3. Chloroform fraction 4. Ethyl-acetate fraction

Table-8. Phytochemical screening of ethanolic (Crude extract), n-hexane, chloroform and ethyl-acetate fractions of *Sarcococca saligna* (D.Don) Muell. Arg.

Phytochemical constituents	Pre-reproductive stage				Reproductive stage				Post-reproductive stage			
	1	2	3	4	1	2	3	4	1	2	3	4
Alkaloids	+	–	+	+	+	–	+	+	+	–	+	+
Anthocyanin and Betacyanins	+	+	+	+	+	+	+	+	+	–	+	+
Anthraquinones	–	–	–	–	–	–	–	–	–	–	–	–
Cardiac glycosides	+	+	–	–	+	–	–	–	+	–	–	–
Flavonoids	+	+	+	+	+	–	+	+	+	–	–	+
Glycosides	–	–	–	–	–	–	–	–	+	–	–	+
Amino acids and Proteins	–	–	–	–	–	–	–	–	+	–	–	–
Reducing sugars	–	–	–	–	+	–	–	–	+	–	–	–
Saponins	+	–	–	+	+	–	–	+	+	–	–	–
Steroids	+	+	+	+	+	+	+	–	+	+	+	–
Tannins	+	–	–	+	+	–	–	+	+	–	–	+
Terpenoids	+	+	+	+	+	–	–	+	+	+	+	–

Key: + Presence, - Absence, 1. Ethanolic crude extract 2. n-hexane fraction 3. Chloroform fraction 4. Ethyl-acetate fraction

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