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INVESTIGATION OF ANTIOXIDANT STATUS OF 29 EDIBLE AND MEDICINAL PLANTS FROM INDIA

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ABSTRACT

Natural antioxidants are widely distributed in flowers. The phytochemicals present in flower exhibit anti-inflammatory, anti-aging, anti-atherosclerosis and anticancer properties. The antioxidant activity of the polyphenols and flavonoids is due to the presence of phenolic hydrogen, which make them hydrogen donating radical scavengers. Among the 29 flowers studied, the phenolic levels ranged from 0.311±0.163mg TAE/g to 1.245±0.163 mg TAE/g. The phenolic levels was highest in *Pelargonium peltatum* (1.245±0.163 mg TAE/g) and lowest in *Bellis perennis* (0.311±0.163mg TAE/g). The flavonoid content was found highest in *Pelargonium peltatum* (0.385±0.001 mg AAE/g) and the lowest in *Rosa hybrid and Celosia cristata* (0.04±0.001 mg AAE/g). The highest antioxidant levels by FRAP assay were found in flower *Pelargonium peltatum* (0.982±0.033 mg AAE/g) and the lowest in *Celosia cristata* (0.118±0.012 mg AAE/g). The coefficient of determination was highest between total antioxidant levels and flavonoid content ($R^2 = 0.7919$) followed by total antioxidant levels and TPC ($R^2 = 0.6124$)

Keywords: Antioxidant, edible and medicinal plants from india

INTRODUCTION

India is a second largest grower of flowers around the world with around 18,000 species of flowering plants. Around 43% of the total flowering plants have potential medicinal value and used as a traditional medicine [1]. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Around 20,000 medicinal plants have been used for medicinal purposes in the Indian subcontinent [2]. Traditionally, flowers constitute an important component of the health care system. In Ayurveda, flowers are used as an alternative medicine in order to treat major conditions [3]. Flowers contain a wide variety of natural antioxidants such as phenolic acid, flavonoids, anthocyanin. Bioactive substances such as carotenoids and phenolic compounds determine the functional properties of edible flower [4]. These compounds have antioxidant properties and inhibit free radicals that induce oxidative stress, reduce the risk of chronic degenerative diseases and cardiovascular diseases. Edible flowers are becoming more popular as they possess the various properties – anti-anxiety, anti-cancer, anti-diabetic, anti-inflammatory, anti-oxidant,

diuretic, immune-modulatory, antimicrobial [5].

Presence of various phenolic acids and flavonoids function as reducing agents and free radical scavengers. Flowers are characterized by the presence of plant phenolic compounds, primarily coumarins and phenylpropanoids that have been shown to possess multiple pharmacological activities. The most widespread of all flavonoids are quercetin, flavonols (3-hydroxyflavones) and flavones, kaempferol, and myricetin [6].

Flowers have been used for years in many cultures. They have traditional or spiritual significance in India. It is worth noticing that the flowers of Indian states have tremendous medicinal properties & are used as herbal medicine in various countries across the world. The aim of the present study was to determine the phenolic, flavonoid and antioxidant properties of various edible flowers from India. Also, the relationship between total phenolic content and antioxidant activity was estimated to enlighten their potential health benefits.

MATERIAL AND METHODS

Processing of flowers

The flowers of *Calliandra haematocephala*, *Caesalpinia pulcherrima*, *Tecoma stans*,

Canna indica, Cassia fistula, Celosia cristata, Hydrangea macrophylla, Allamanda cathartica, Euphorbia milii, Plumeria rubra, Vachellia nilotica, Plumbago auriculata, Viola x wittrockiana, Butea monosperma, Pelargonium peltatum, Ixora coccinea, Senna sulfurea, Petunia hybrida, Dahlia, Tropaeolum majus, Tagetes erecta, Bougainvillea glabra, Calendula officinalis, Chrysanthemum morifolium, Hibiscus rosa-sinensis, Bellis perennis, Rosa hybrid, Rosa hybrid tairianca, Dianthus chinensis were collected and dried under sun for 3-5 days. The dried flowers were powdered and stored at room temperature.

Sample extraction

1 g dried flowers was weighed into a beaker and 100 ml of boiling distilled water was added. After brewing for 5 min, the blend was removed and the extract was cooled down. All analyses of aqueous extracts were done in triplicate.

Estimation of total phenol content (TPC)

The total phenol content (TPC) was determined by spectrophotometer using tannic acid as standard with some modifications [7]. 1.0 ml of the diluted sample extract (in triplicate) was added to tubes containing 5.0 ml of 1/10 dilution of Folin Ciocalteu's reagent in water. Then, 4.0

ml of a sodium carbonate solution (7.5% w/v) was added and incubated at room temperature for one hour. The absorbance was measured at 765 nm. The total phenolic content was calculated from the calibration curve and the results were expressed as mg of tannic acid equivalent per g dry weight (mg TAE/g).

Determination of Total flavonoid content

Total flavonoid content (TFC) was measured by the modified aluminium chloride colorimetric assay [7]. The reaction mixture consisted of 1 ml of extract and 4 ml of distilled water taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was added and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was added and final volume of the mixture was brought to 10 mL with double-distilled water. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was calculated from the calibration curve and was expressed as mg Ascorbic acid equivalent AAE/g of extract.

Determination of antioxidant power by using modified ferric ion reducing antioxidant power assay (FRAP)

The total antioxidant capacity was determined spectrophotometrically using ascorbic acid as standard by modified FRAP assay [7]. 0.1 ml of extract was taken and to it 0.9 ml of ethanol, 5 ml of distilled water, 1.5 ml of HCl, 1.5 ml of potassium ferricyanide, 0.5 ml of 1 % SDS and 0.5 ml of 0.2 % of ferric chloride was added. This mixture was boiled in water bath at 50°C for 20 minutes and cooled rapidly. Absorbance was measured at 750 nm to measure the reducing power of the tea extract. The antioxidants in samples were derived from a standard curve of ascorbic acid and were expressed as mg ascorbic acid equivalent (AAE)/ g.

Statistical analysis

The assays were carried out in triplicate and the results were expressed as mean values and the standard deviation (SD). The statistical differences were done by one way ANOVA ($p \leq 0.05$). Correlation coefficient (R) and coefficient of determination (R^2) were calculated using Microsoft Excel 2007.

RESULT AND DISCUSSION

Among the 29 flowers studied, the phenolic levels ranged from 0.311 ± 0.163 mg TAE/g to 1.245 ± 0.163 mg TAE/g (Table 1). The phenolic levels was the highest in *Pelargonium peltatum* (1.245 ± 0.163 mg

TAE/g) followed by *Senna sulfurea* (1.00 ± 0.187 mg TAE/g), *Plumbago auriculata*, *Viola x wittrockiana*, *Butea monosperma* (0.975 ± 0.219 mg TAE/g to $.995 \pm 0.23$ mg TAE/g) followed by *Bougainvillea glabra* (0.845 ± 0.112 mg TAE/g), *Calendula officinalis*, *Dahlia*, *Tagetes erecta*, *Vachellia nilotica*, *Ixora coccinea* (0.785 ± 0.23 mg TAE/g to 0.700 ± 0.202 mg TAE/g). Amongst the flowers having low phenolic levels are *Euphorbia milii*, *Celosia cristata*, *Canna indica*, *Cassia fistula*, *Chrysanthemum morifolium*, *Allamanda cathartica*, *Petunia hybrid*, *Rosa hybrid*, *Hibiscus rosa-sinensis*, *Rosa hybrid tairrianca*, *Tecomastans* (0.500 ± 0.022 mg TAE/g to 0.43 ± 0.202 mg TAE/g). The lowest phenolic levels were found in *Bellis perennis* (0.311 ± 0.163 mg TAE/g).

The flavonoid contents in various flower was found highest in *Pelargonium peltatum* (0.385 ± 0.001 mg AAE/g) and the lowest in *Rosa hybrid* and *Celosia cristata* (0.04 ± 0.001 mg AAE/g) (Table 1). The *Butea monosperma*, *Viola x wittrockiana*, *Plumeria rubra* had phenolic levels in the range of 0.2835 ± 0.0015 mgAAE/g to 0.239 ± 0.003 mgAAE/g followed by *Canna indica*, *Senna sulfurea*, *Tropaeo lummajus* (0.132 ± 0.026 to 0.135 ± 0.018 mg AAE/g). The *Calliandra haematocephala*, *Hibiscus*

rosa-sinensis, *Calendula officinalis*, *Rosa hybrid tairrianca*, *Canna indica*, *Bougainvillea glabra*, *Chrysanthemum morifolium*, *Euphorbia milii*, *Dianthus chinensis*, *Ixora coccinea* have low flavonoid levels ($.095 \pm 0.012$ mg AAE/g to 0.067 ± 0.033 mgAAE/g). The lowest flavonoid levels were found in *Petunia hybrid*, *Rosa hybrid*, *Celosia cristata* ($0.04 \text{mg} \pm 0.015$ AAE/g).

Among the 29 flowers studied the antioxidant levels ranged from 0.982 ± 0.033 mg AAE/g to 0.118 ± 0.012 mg AAE/g (Table 1). The highest antioxidant levels by FRAP assay were found in flower *Pelargonium peltatum* (0.982 ± 0.033 mg AAE/g) and the lowest in *Celosia cristata* (0.118 ± 0.012 mg AAE/g). The *Butea monosperma*, *Viola x wittrockiana*, *Senna sulfurea*, *Pelargonium peltatum*, *Bougainvillea glabra*, *Ixora coccinea*,

Tagetes erecta had antioxidant level in the range of 0.533 ± 0.015 mgAAE/g to 0.300 ± 0.013 mgAAE/g. The flowers like *Caesalpinia pulcherrima*, *Vachellia nilotica*, *Calliandra haematocephala*, *Canna indica*, *Dahlia*, *Tecoma stans*, *Chrysanthemum morifolium*, *Plumbago auriculat*, *Hydrangea macrophylla*, *Allamanda cathartica*, have low antioxidant in the range of (0.292 ± 0.389 to 0.197 ± 0.011 mg AAE/ g). The correlations between antioxidant activity and total phenolics content, total flavonoid content and antioxidant activity were determined by linear regression analysis in 29 flowers studied. The coefficient of determination was highest between total antioxidant levels and flavonoid content ($R^2 = 0.7919$) (Figure 2) followed by total antioxidant levels and TPC ($R^2 = 0.6124$) (Figure 1).

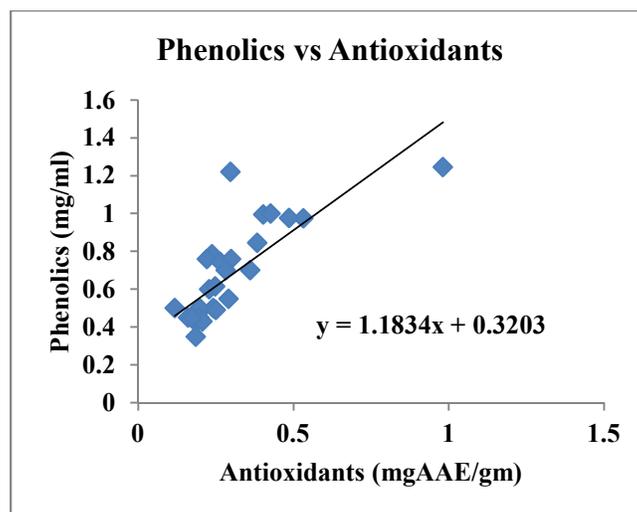


Figure 1: Linear correlations between phenolic content and antioxidants levels. Coefficient of determination (R^2) = 0.6124

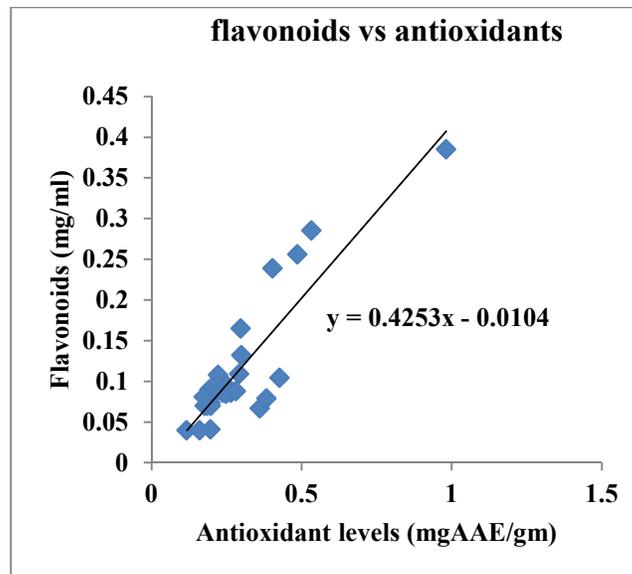


Figure 2 : Linear correlations between Flavonoid content and antioxidants levels ; Coefficient of determination (R^2) = 0.7919

Table 1: Total Phenolic content, Total Flavonoid content and Total Antioxidant in edible flowers

S. No.	Flowers	Total Phenolic content (mg TAE/g)	Total Flavonoid content (mg AAE/g)	Total antioxidant (mg AAE/ml)
1)	<i>Calendula officinalis</i>	0.785	0.086	0.238
2)	<i>Hibiscus rosa-sinensis</i>	0.475	0.09	0.194
3)	<i>Ixora coccinea</i>	0.7	0.067	0.361
4)	<i>Tropaeolum majus</i>	0.6	0.1035	0.229
5)	<i>Allamanda cathartica</i>	0.44	0.074	0.197
6)	<i>Bellis perennis</i>	0.35	0.07	0.186
7)	<i>Bougainvillea glabra</i>	0.845	0.079	0.384
8)	<i>Butea monosperma</i>	0.975	0.2853	0.533
9)	<i>Caesalpinia pulcherrima</i>	0.55	0.1092	0.292
10)	<i>Calliandra haematocephala</i>	0.5	0.095	0.248
11)	<i>Canna indica</i>	0.49	0.086	0.265
12)	<i>Cassia fistula</i>	0.45	0.076	0.191
13)	<i>Celosia cristata</i>	0.5	0.04	0.118
14)	<i>Chrysanthemum morifolium</i>	0.44	0.073	0.193
15)	<i>Dahlia</i>	0.76	0.108	0.222
16)	<i>Dianthus chinensis</i>	0.44	0.07	0.178
17)	<i>Euphorbia milii</i>	0.5	0.07	0.198
18)	<i>Hydrangea macrophylla</i>	0.745	0.087	0.265
19)	<i>Pelargonium peltatum</i>	1.245	0.385	0.982
20)	<i>Petunia hybrida</i>	0.45	0.041	0.197
21)	<i>Plumbago auriculata</i>	0.995	0.239	0.404
22)	<i>Plumeria rubra</i>	0.615	0.085	0.249
23)	<i>Senna sulfurea</i>	1	0.1044	0.427
24)	<i>Tagetes erecta</i>	0.76	0.132	0.3
25)	<i>Tecoma stans</i>	0.43	0.085	0.208
26)	<i>Vachellia nilotica</i>	0.7	0.085	0.282
27)	<i>Viola x wittrockiana</i>	0.976	0.256	0.487
28)	<i>Rosa hybrid</i>	0.4501	0.04	0.161
29)	<i>Rosa hybrid tair rianca</i>	0.4502	0.081	0.175

DISCUSSION

Antioxidants play an important role in protecting cellular damage by reactive oxygen species. The flowers contain various phytochemicals such as quinones, saponins, triterpenoids, flavonoids, phenols, sterols, glycosides, tannins, furanoids and small amounts of sugars. The phytochemicals present in flowers are free radical quencher and a potent source of natural antioxidants and antimicrobial agents, which justifies their traditional use in green therapeutics.

The flavonoids such as rutin, quercetin glucoside, and quercetin rhamnoside present in *flowers* function as free radical scavengers [8]. In *Pelargonium peltatum*, the major flavonoids present are myricetin, quercetin, kaempferol and their glucosides in addition to methylated flavonoids and anthocyanins [9]. The flavonoids present in flowers have wide range of anti-inflammatory, anti-oxidant, anti-microbial, and anti-cancer properties. Flavonoids of the flowers have various functions such as free radical scavenging, inactivation of peroxides and other reactive oxygen species, chelation of metals and quenching of secondary lipid oxidation products. They are also known to be potent inhibitors for several enzymes, such as aldose reductase, Ca²⁺-ATPase, xanthine oxidase

(XO), cyclo-oxygenase (COX), lipoxygenase and phosphoinositide 3-kinase [10]. The flavonoids such as Hesperidin, Luteolin and Quercetin present in various flowers are known to possess anti-inflammatory property. These flavonoids play an important role in preventing inflammation involved diseases such as leukemia, sepsis, asthma, sclerosis, atherosclerosis, psoriasis, allergic rhinitis, ileitis/ colitis, rheumatoid arthritis, etc. has been proposed [11, 12]. Various flavonoids such as apigenin, galangin, flavone and flavonol glycosides, isoflavones, flavanones, and chalcones have antimicrobial activity [13]. The antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, and so forth. Lipophilic flavonoids may also disrupt bacterial membranes [14].

The high content of phenolic compounds in the flower extract might explain its higher antioxidant capacity compared to the leaf and stem extract. The phenolic contents of flowers possess enzyme inhibitory and antioxidant activities and are responsible for their therapeutic effects in traditional medicine [15]. The high phenolic levels observed in flowers could be due to presence of phenols such as chlorogenic acid and 3, 5-dicaffeoylquinic acid. The phenolic

acid present in flowers have a important property of inhibiting α -glucosidase and α -amylase, which are key enzymes and responsible for the digestion of dietary carbohydrates to glucose. Thus, flowers based products are polyphenol rich which can modulate carbohydrate and lipid metabolism, hyperglycemia, dyslipidemia and insulin resistance, improve β -cell function, stimulate insulin secretion, improve adipose tissue. These compounds can also prevent the development of long-term diabetes complications, including cardiovascular disease, neuropathy, nephropathy and retinopathy [16].

The high antioxidant activity in various flowers could be due to phytochemicals such as flavonoids,

especially quercetin and rutin [17]. The high antioxidant activity observed in *Pelargonium peltatum*, *Petunia hybrid*, *Viola x wittrockiana* is due to presence of high anthocyanin content as reported earlier [18]. Anthocyanins are pigments abundantly present in nature, which play very important roles in basic plant processes such as propagation and defence mechanisms against external stress factors. However *V. vittrockiana* and *T. majus* also have high antioxidant activity, which could be attributed to presence of vitamin C [19], polyphenols [17], carotenoids [20], chlorogenic acid and not anthocyanin content [18]. The antioxidant level reported in *Tagetes erecta* could be due to high xanthophyll (lutein) content [21].

Table 2: List of phytochemicals present in flowers

S. No	Flowers	Main Phenolic compounds	Main Flavonoid compounds	References
1)	<i>Calendula officinalis</i>	Xanthones, Stilbene, Quinones, Phenolic acids, Coumarins, Acetophenones.	Quercetin, Isorhamnetin, Narcissin, Calendoflavoside.	22
2)	<i>Hibiscus rosa-sinensis</i>	Kaempferol-7-O-[6''-O-p-hydroxybenzoyl- β -D-glucosyl-(1-6)- β -D-glucopyranoside], scutellarein-6-O- α -L-rhamnopyranoside-8-C- β -D-glucopyranoside.	Quercetin-3-diglucoside, 3,7-diglucoside, Cyanidin-3,5-diglucoside.	23
3)	<i>Ixora coccinea</i>	Kaempferol, luteolin, Phenolic acids, Gallic acid, Caffeic acid, Ferulic acid.	Anthocyanin, proanthocyanins, glycoside of kaempferol	24
4)	<i>Tropaeolum majus</i>	Gallic acid, Caffeic acid, Quercetin, Ferulic acid, Resveratrol	Anthocyanin, pelargonidin 3-sophoroside, flavones.	21
5)	<i>Allamanda cathartica</i> .	Phenolic acids, Gallic acid, Caffeic acid, Ferulic acid, Resveratrol, Coumarins.	Isoflavonoids, isoflavan-3-ene, coumestane	25
6)	<i>Bellis perennis</i>	Caffeic acid, ferulic acid, sinapic acid, p- coumaric acid and salicylic acid.	Flavone, aurone, auronols	26
7)	<i>Bougainvillea glabra</i>	Gallic acid, syringic acid, p-hydroxybenzoic acid, Procatechuic acid, Vanillic acid, Ferulic acid, Sinapic acid, Caffeic acid, Coumaric acid, Chlorogenic acid	Isorhamnetin-glucorhamnoside, Quercetin-xyloside, isovitexin, vitexin, kaempferol,	27
8)	<i>Butea monosperma</i>	Phenolic acids, Gallic acid, Caffeic acid, Ferulic acid, Coumarins, Acetophenones	flavone, 7,3'4'-trihydroxyflavone, Isomonospermoside, butin, isoflavones, liquiritigenin.	28
9)	<i>Caesalpinia pulcherrima</i>	Methyl gallate, 6-O-galloyl-D-glucoside, Gentisic acid 5-O- α -D-(6'-O-galloyl)glucopyranoside,	Apigenin, luteolin, quercetin, kaempferol, myricetin	29

		Gallocatechin, Myricetin 3-rhamnoside and ampelopsin.		
10)	<i>Calliandra haematocephala</i>	Xanthones, Stilbene, Quinones, Phenolic acids, Coumarins, Acetophenones.	Quercitrin 2"-O-caffeate, Quercitrin 3"-O-gallate, isoquercetin, myricetin.	30
11)	<i>Canna indica</i>	- Phenolic acids, Gallic acid, Caffeic acid, Ferulic acid, Coumarins, Acetophenones, sinapic acid	Quercitrin, anthocyanin, isoflavones	31
12)	<i>Cassia fistula</i>	Phenolic acids, Gallic acid, Quercetin, Coumarins, Coumarins, Acetophenones, Caffeic acid, Ferulic acid, Kaempferol, penta hydroxyl flavanox anthraquinone	Proanthocyanin (flavan-3-ol), kaempferol, epicatechin.	32
13)	<i>Celosia cristata</i>	Phenolic acids, Gallic acid, Caffeic acid, Ferulic acid, Phenolic acids, Coumarins, Acetophenones	Betalains, anthocyanidins, isoflavones	33
14)	<i>Chrysanthemum morifolium</i>	phenolic acids, Gallic acid, Quercetin, Coumarins, Acetophenones, Caffeic acid, Ferulic acid, Kaempferol	Vitexin-2-O-rhamnoside, quercetin-3-galactoside, luteolin-7-glucoside, quercitrin.	34
15)	<i>Dahlia</i>	Hydroxycinnamic acids, coumarin, Caffeic acid, Ferulic acid, Kaempferol, Gallic acid	Anthocyanin, chalcones, flavanones, isoflavones	35
16)	<i>Dianthus chinensis</i>	Phenolic acids, Gallic acid, Caffeic acid, Ferulic acid, Phenolic acids, Coumarins, Acetophenones, sinapic acid	Kaempferol 3-O-beta -d-glucopyranosyl, Beta -d-glucopyranoside, O-flavonoids.	36
17)	<i>Euphorbia milii</i>	Phenolic acids, Gallic acid, Quercetin, Coumarins, Acetophenones, Caffeic acid, Ferulic acid, Kaempferol, Rosmarinic acid, Protocatechuic acid	Anthoxanthins, flavone (2-phenyl-4-chromone),	37
18)	<i>Hydrangea macrophylla</i>	Phenolic acids, Coumarins, Acetophenone, Phenolic acids, Gallic acid, Caffeic acid, Ferulic acid.	Luteolin, apigenin, and tangeritin.	38
19)	<i>Pelargonium peltatum</i>	Gallic acid, Caffeic acid, Ferulic acid, Phenolic acids, Coumarins, Acetophenones, sinapic acid	3,5-diglucoside, 3-glucoside-5-(acetyl)glucoside of pelargonidin, cyaniding, peonidin.	39
20)	<i>Petunia hybrida</i>	3,4-dihydroxyphenylacetic acid, caffeic acid, Phenolic acids, Gallic acid.	Anthocyanin, cyanidin, delphinidin.	40
21)	<i>Plumbago auriculata</i>	Gallic acid, Caffeic acid, Ferulic acid, Phenolic acids, Coumarins, Acetophenones, sinapic acid, Xanthones, Stilbene, Quinones	Quercetin, anthocyanin, neoflavonoids.	41
22)	<i>Plumeria rubra</i>	Kaempferol, luteolin, Xanthones, Stilbene, Quinones, Phenolic acids, Coumarins, Acetophenone, Phenolic acids	Quercetin, rubrinol glycoside, flavanone glycoside, rubranin	43
23)	<i>Senna sulfurea</i>	Gallic acid, Caffeic acid, Ferulic acid, Phenolic acids, Coumarins, Acetophenones, sinapic acid, Quinones	Anthraquinones, chrysophanol, physcione and quercetine	44
24)	<i>Tagetes erecta</i>	Phenolic acids, Gallic acid, Quercetin, Coumarins, Acetophenones, Caffeic acid, Ferulic acid, Kaempferol	Quercetagenin 6,3'-dimethy ether, quercetagenin 7-methy ether, quercetin	45
25)	<i>Tecoma stans</i>	Chlorogenic, Caffeic, Vanillic, O-cumaric	Anthraquinones, flavan-3-ols, isoflavonols.	46
26)	<i>Vachellia nilotica</i>	Kaempferol, luteolin, Phenolic acids, Gallic acid, Caffeic acid, Ferulic acid, Resveratrol, Xanthones, Stilbene, Quinones, Phenolic acids, Coumarins, Acetophenones	flavaone, quinolinone, quercetin	47
27)	<i>Viola x wittrockiana</i>	Phenolic acids, Gallic acid, Caffeic acid, Ferulic acid, Resveratrol, Quinones, Phenolic acids,	Quercetin, luteoline, flavone.	48
28)	<i>Rosa hybrid</i>	Phenolic acids, Gallic acid, Caffeic acid, Ferulic acid, Resveratrol, Xanthones, Stilbene, Quinones, Phenolic acids, Coumarins, Acetophenones	Anthocyanidin 3,5-O-diglucosides, anthocyanidin 3-O- glucosides, flavonols	49
29)	<i>Rosa hybrid tairrianca</i>	Phenolic acid, Caffeic acid, Gentisic acid, protocatechuic acid, gallic acid, salicylic acid, sinapic acid and p- coumaric acid, Quinones.	Anthocyanin, flavonols, flavan-3-ols	49

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