Studies on biological activities and phytochemical composition of *Hibiscus* species- A review

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Abstract: This article reviews the antimicrobial and antioxidant activities as well as the phytochemical composition of extracts from some *Hibiscus* species. Some of the bioactive constituents of these plants were isolated, purified and analyses for possible use in making drugs. Thus these plants have great medicinal potential for the therapy of infection.


Keywords: *Hibiscus*; biological activities; phytochemical composition; extract

1. Introduction

Plants contain secondary metabolites, which are organic compounds that are not directly involved in the normal growth, development, or reproductions of organisms but often play an important role in plant defenses (Harborne and Baxter, 1993). Examples include alkaloids, glycosides, terpenoids, phenols, tannins, flavonoids and saponins (Edema and Alaga, 2012). Furthermore, there is growing interest in the chemical composition of plants towards discovery of more effective bio-therapeutic agents (Roa and Rao, 2002). The primary benefit of using plant-derived medicines is that they are readily affordable and accessible (Grunwald, 1995).

Continuous exposure to chemicals and contaminants leads to increase the free radicals amount and causes irreversible oxidative damage including biological damage, DNA damage, diabetes, respiratory tract disorders, carcinogenesis and cellular degeneration related to ageing (Tseng *et al.*, 1997).

The *Hibiscus* genus (Malvaceae) contains several species, many of which have been used medicinally and is comprises of about 275 species in the tropics and sub-tropics and most *Hibiscus* species have a remarkable color pattern with the base of corolla forming a deep-colored heart (Lowry, 1976).

Leaves and flowers of selected *Hibiscus* species were evaluated for antioxidant, antityrosinase and antibacterial activities. Leaves of *H. tiliaceus* had the strongest antityrosinase activity and have potentials to be developed into functional food and skin care products. At 1 mg extract/disc, leaves of *H. sabdariffa* were found to inhibit Gram-positive bacteria of *Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus*. At 2 mg extract/disc, leaves of *H. sabdariffa* inhibited both Gram-positive and Gram-negative bacteria of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella choleraesuis* (Wong *et al.*, 2010). Stems and roots of *H. taiwanensis* have been used as anti-inflammatory, antifungal, antipyretic, and anthelmintic agents (Wu *et al.*, 2005). Flowers of *H. tiliaceus* L. are widely used for birth control and for treated skin infections (Rosa *et al.*, 2006).

The auto-oxidation of unsaturated lipids is caused by free radical (Ak and Gülçin, 2008) and the antioxidants are used to intercept the free radical chain of oxidation and donate hydrogen from the phenolic hydroxyl groups and forming a stable end product (Jain *et al.*, 2008). It was shown that NO plays a crucial role in the pathogenesis of inflammation where it is secreted as inflammatory mediator, this may explain the use of *H. rosa* and *H. cannabinaus* extracts for the treatment of inflammatory disease (Lee *et al.*, 2007) and may be due to some various active compounds including tannins, polyphenolics, alkaloids, essential oils and steroids which inhibited NO production by radical scavenging activity.

On the other hand, the level of nitric oxide was significantly reduced by *H. rosa* extract whereas it scavenged up to 36.3% nitric oxide radicals at a concentration of 500 µg/ml (Abdel Ghaffar and El-Elaimy, 2012). This study would contribute additional knowledge on the antioxidant and bioactivities of selected some species of *Hibiscus*.

2. Biological activity of some *Hibiscus* species

A summary of certain screening studies related to the antimicrobial and antioxidant activities of *Hibiscus* species are presented in Table 1. DPPH method was found to be used mostly for the in vitro antioxidant activity evaluation purpose.
<table>
<thead>
<tr>
<th>Hibiscus species</th>
<th>Part and extract used</th>
<th>Bioactivity</th>
<th>Bioassay methods</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H. rosa-sinensis</strong></td>
<td>90% methanolic leaf extract.</td>
<td>Anticancer and antioxidant activities.</td>
<td>MTT reduction assay, FRAP assay, DPPH activity, Superoxide dismutase activity.</td>
<td>Divya <em>et al.</em> (2013)</td>
</tr>
<tr>
<td></td>
<td>Distilled water and ethanol (99.7%) flower extract.</td>
<td>Antioxidant and antibacterial activities.</td>
<td>DPPH radical scavenging activity and ferric reducing antioxidant power assay (FRAP). Agar disk diffusion method.</td>
<td>Mak <em>et al.</em> (2013)</td>
</tr>
<tr>
<td></td>
<td>Methanolic extract of leaves, stem and root of five cultivars (Red, Yellow, Orange, Pink and White)</td>
<td>Antioxidant Activity.</td>
<td>DPPH radical scavenging activity, Reducing power assay, phosphomolybdenum method.</td>
<td>Patel <em>et al.</em> (2012)</td>
</tr>
<tr>
<td></td>
<td>70% ethanol/water leaves extract.</td>
<td>Antioxidant Activity.</td>
<td>Free radical scavenging activity, Lipid peroxidation (LPO) and protein oxidation (PO)</td>
<td>Abdel Ghaffar and El-Elaimy (2012)</td>
</tr>
<tr>
<td></td>
<td>Dried flower soaked in cold water.</td>
<td>Antibacterial activity</td>
<td>Agar disc diffusion, agar well diffusion methods.</td>
<td>Ruban and Gajalakshmi (2012)</td>
</tr>
<tr>
<td></td>
<td>Calyx in methanol, ethanol, acetone and water extract</td>
<td>Antioxidant activity.</td>
<td>(DPPH) inhibition and lipid peroxidation inhibition.</td>
<td>Anokwuru <em>et al.</em> (2011)</td>
</tr>
<tr>
<td></td>
<td>Extracts of dried calyx and fruit with distilled water ethanol (30, 60 and 95%)</td>
<td>Antioxidant capacity</td>
<td>DPPH, ABTS assays.</td>
<td>Yang <em>et al.</em> (2012)</td>
</tr>
<tr>
<td><strong>H. mutabilis</strong></td>
<td>Chloroform and methanolic leaf extracts.</td>
<td>Antioxidant activity.</td>
<td>DPPH</td>
<td>Moussa <em>et al.</em> (2011)</td>
</tr>
<tr>
<td><strong>H. tiliaceus</strong></td>
<td>Ethanol extract of the dried leaves</td>
<td>Antioxidant, Antimicrobial activities.</td>
<td>Disc diffusion method, DPPH</td>
<td>Ramproshad <em>et al.</em> (2012)</td>
</tr>
</tbody>
</table>

MTT: Methyl Trizoly tetrazolin; FRAP (Ferric Reducing Ability of plant; DPPH (1, 1-Diphenyl -2-picryl- hydrazyl)
2.1. Biological activity and chemical composition of Hibiscus rosa-sinensis

Previous studies have been indicated that H. rosa-sinensis had to possess bioactive properties and is recommended to be used as an herbal alternative to cure many diseases (Obi et al., 1998). In a study of Shivananda et al. (2007), the in vitro antibacterial activity as the same as the wound-healing activity of the ethanol extracts of H. rosa-sinensis flowers in vivo. Flowers and leaves are found to possess antioxidant, anti-inflammatory, anti-infectious, antimicrobial, anti-inflammatory, anti-diarrheic and antipyretic activity (David and Leonard, 1998).

Phytochemicals like tannin, phlobatannins, cardiac glycosides, flavonoids, terpenoids, saponins and others are present in leaves, stem and root of the plant (Patel et al., 2012). Flowers contain anthocyanins, which may be responsible for its antioxidant effects (Gauthaman et al., 2006). A number of previous studies reported that H. rosa-sinensis contains flavonoids, cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin, ascorbic, citric, tartaric and oxalic acid (Shukla and Mishra, 2001). Recently, four new phytoconstituents (n-hexacosa-3-one-20, 21-diol, n -triacontane n -triacontan-15-one and n – hentriacontane) have been isolated from the alcoholic extracts of leaf and flower (Siddiqui et al., 2006). The leaf extract exhibited significant antioxidant and anticancer activities due to the increased flavonoids and terpenoids level and the phytochemical analyses indicated the constituents presented (flavonoids, terpenoids, saponins, tannins and glycosides) are responsible for pharmacological effects (Abdel Ghaffar and El-Elaiby, 2012; Divya et al., 2013). The aqueous and ethanolic extracts of hibiscus flowers at the concentration of 100 and 50 mg/mL were shown good inhibition zones (IZs) against the growth of Salmonella typhimurium and S. aureus, respectively, and the IZs ranged from 9–14 mm (Mak et al., 2013).

The flower extracts had stronger antibacterial effects than that of leaves at the applied doses of 50 and 100 mg/well and raises the possibility of using the extracts as antibacterial agents in treating pathological conditions caused by S. aureus and S. typhimurium infection (Uddin et al., 2010). Chemically, flowers have been reported to contain cyaniding diglucoside, flavonoids and vitamins - thiamine, riboflavin, niacin and ascorbic acid; leaves contained beta sitosterol, sigma sterol, taraxerol, acetate and three cyclopropane compounds and their derivatives (Patel et al., 2012). The cold extraction illustrates maximum IZs against B. subtilis, E. coli viz., (17.00 ± 2.91), (14.50 ± 1.71) mm, followed by hot extraction against, E. coli, Salmonella sp. as (11.66 ± 3.14), (10.60 ± 3.09) mm. The highest IZs recorded in methanol extract against B. subtilis, E. coli as (18.86 ± 0.18), (18.00 ± 1.63) mm pursued by ethanol extraction showed utmost IZs recorded against Salmonella sp. at (20.40 ± 1.54) mm. On the other hand, the flower crude protein showed a maximum inhibitory zone observed against Salmonella sp., E. coli viz., (16.55 ± 1.16), (14.30 ± 2.86) mm. Consequently, Ruban and Gajalakshmi (2012) reported that the flower material can be taken as an alternative source of antibacterial agent against the human pathogens. The flower crude extracts was reported to contain flavonoids, tannins, alkaloids and triterpenoids, all of which are known to have antibacterial affects (Scalbert, 1991). It supports the earlier investigation that the tannins isolated from the flower possess remarkable toxic activity against bacteria and may assume pharmacological importance (Salem et al., 2013; Kannathasan et al., 2011).

Pharmacologically, leaves, stem and root of H. rosa-sinensis contain a remarkable quantities of flavonoids which are associated with antioxidant, fever-reducing, pain-relieving and spasm-inhibiting activities and the flower has soothing properties which are used to relieve menstrual cramps and relax spams and general cramping (Patel et al., 2012) and treating inflammations (Sotheeswaran et al., 1998). Saponins in H. rosa-sinensis bind to cholesterol to form insoluble complexes and excreted via the bile and reduces blood pressure and have been found to be potentially useful for the treatment of hypercholesterolemia (Olaleye, 2007). This plant is used to soothe irritated tissues and the mucous membranes that line the respiratory tract, which eases hacking coughs and other respiratory ailments due to the presence of terpenoids (Nayak et al., 2007).

2.2. Biological activity and chemical composition of Hibiscus sabdariffa L.

Sorrel (H. sabdariffa), a medicinal herb commonly uses to make drink and pickle, is used in folk medicine in the treatment of hypertension, liver diseases, and fever (Wang et al., 2000; Akindahunsi and Olaleye, 2003; Odigie et al., 2003). H. sabdariffa is reported to possess antihypertensive, antioxidant, anti-cancer, anticlastrogenic, hypolipidaemic, hepatoprotective, anti-stress, antispasmodic, diuretic and anti diarrheal activities (Joshi and Parle, 2006). The decoction of the seeds is given to augment or induce lactation in poor letdown and maternal mortality (Okasha et al., 2008). Furthermore, the ethanol extract of leaves showed an antioxidant
activity at the concentration of 0.13 mg/ml (Mungole and Chaturvedi, 2011) and the presence of phenols may be responsible for this activity.

The oil is consists of linoleic/oleic category; the global characteristics of oil suggest that it could have important industrial applications (Essa et al., 2007). The acute and sub-chronic toxicity studies characterize the plant to have low toxicity which makes it safe for human consumption (Okasha et al., 2008). Fullerton et al. (2011) found that the overall mean IZs for 80% aqueous methanol extract was 12.66 mm for 10%, 10.75 mm for 5%, and 8.9 mm for 2.5%. The highest IZs were observed in veterinary samples (11.16 mm), and the lowest in the food samples against E. coli O157:H7 (10.57 mm). These findings indicated that H. sabdariffa was effective at all levels in inhibiting E. coli O157:H7; thus it possesses antimicrobial activity and hold great promise as an antimicrobial agent (Fullerton et al., 2011). Nair and Chanda (2006) also found similar effects, and they reported that standard ATCC strains of Gram-positive bacteria were more sensitive than Gram-negative ones toward the plant extracts. The results of antibacterial activity of gossypetin isolated from H. sabdariffa revealed that the activity may be due to polyphenolic nature of the flavonoid gossypetin (Mounnissamy et al., 2002). The mechanism of action may be by inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the bacterial cells (Walsh et al., 2003). These processes include the inhibition of electron transport, protein translocation, phosphorylation steps, and other enzyme-dependent reactions. Nair and Chanda (2006) showed inhibition at concentrations as low as 9.75 µg/mL. The minimum inhibitory concentrations (MICs) of 0.30±0.2–1.30±0.2 mg/mL were exhibited against S. aureus, B. stearothermophilus, M. luteus, Serratia marcescens, Clostridium sporogenes, E. coli, Klebsiella pneumoniae, B. cereus, and P. fluorescence (Nair and Chanda 2006).

The juice extracts showed significant (P<0.05) antimicrobial activities against E. coli, S. typhi and Candida albicans, implying that the juices possess both antibacterial and antifungal properties (Edema and Alaga, 2012). Cowan (1999) suggested the antimicrobial action may be attributed to the intrinsic properties that are related to the permeability of their cell surface to the extracts. The antibacterial activity of roselle extracts against E. coli, S. aureus, Streptococcus mutans and P. aeruginosa, showed varying degrees of inhibition on the tested organisms (Al-Hashimi, 2012). Aqueous-methanolic extract of H. sabdariffa L. calyces have been found to exhibit antibacterial activities against S. aureus, B. stearothermophilus, M. luteus, S. mascalces, C. sporogenes, E. coli, K. pneumoniae, B. cereus and P. fluorescence (Olaleye, 2007). Antibacterial effects of this plant extract against E. coli, P. aeruginosa and S. aureus suggest that they may possess remarkable therapeutic action in the treatment of gastrointestinal infection and diarrhea in man and skin diseases (Rogger et al., 1990).

H. sabdariffa seed extract are characterized by a very low degree of toxicity with LD₅₀ of above 5000 mg/kg in rats (Bako et al., 2009). The methanol and ethanol were better solvents for the extraction of phenols of H. sabdariffa calyx compared to water and acetone and phenols contributed more to the antioxidant activity compared to flavonoids (Anokwuru et al., 2011). The aqueous extracts from H. sabdariffa had possessed a potent protective effect against the oxidative stress induced by sub lethal dose of Malathion on the rat kidney (Mossalam et al., 2011). Analysis of the extract revealed the presence of alkaloids (0.08%), tannins (0.18%), saponins (1.46%), flavonoids (2.41%), phenols (0.08%) and glycosides (0.13 %) (Edema and Alaga, 2012).

The biological activity of these chemical groups had been reported in related plants with potential medicinal properties (Fasola, 2000). These chemical compounds are known to display inhibitory activities against many microorganisms (Onyilaga and Shahidul, 2009). The effect of aqueous extract of H. sabdariffa on the rate of crystallization of calcium oxalate crystals in kidney was higher than the ethanol extract followed by chloroform extract (Saleh et al., 2013). Also Roselle anthocyanins can be applied in a variety of food products as food colourants such as confectionery products, gelatin desserts, snacks, cake, pudding, ice cream and beverages (Abou-Arab et al., 2011).

Table 2 presents the chemical constituents found in different parts of H. sabdariffa. The phenolic content in the plant consists mainly of anthocyanins like delphinidin-3-glucoside, sambubioside, and cyanidin-3-sambubioside; other flavonoids like gossypetin, hibiscetin, and their respective glycosides; protocatechuic acid, eugenol, and sterols like β-sitosterol and ergosterol (Ali-Bradeldin et al., 2005). Roselle calyx extract is a good source of antioxidants from its anthocyanins (Ajiboye et al., 2011). Anthocyanin is one type of flavonoid component that can be in Roselle calyces (Tsai et al., 2002). Additionally, the anthocyanin is the major source of antioxidant capacity in roselle petale extract (Tsai et al., 2002). Previous study reported that the aqueous extract of this plant could inhibit several nosocomial infectious bacteria such as methicillin-resistant S. aureus and K. pneumoniae (Liu et al., 2005). The leaf is reported to contain protein, fat, carbohydrate, fibre, ash, calcium,
phosphorus, iron, thiamine, β-carotene, riboflavin, niacin and ascorbic acid (Ajiboye et al., 2011). The plant contains flavonoids such as hibiscitrin and hibiscetin and dried calyces contain the flavonoids gossypetine, hibiscetine and sabdaretine. It also contains alkaloids, β-sitosterol, anthocyanin, citric acid, cyanidin-3-rutinoside, delphinidin, galactose, pectin, protocatechuic acid, quercetin, steaic acid and wax (Vilasinee et al., 2005). Small amounts of delphinidin 3-monoglicoside, cyanidin 3-monoguloside (chrysanthenin) and delphinidin are also present. Three water soluble polysaccharides have been isolated from flower buds; neutral polysaccarides composed of arabinans and arabinogalactans (Muller and Franz, 1992).

Table 2: Phytochemicals of H. sabdariffa

<table>
<thead>
<tr>
<th>Part of the plant</th>
<th>Chemical constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower</td>
<td>Carbohydrates, arabinans, mannose, sucrose, thiamin, xylose, mucilage, niacin, pectin, proteins, fat, arabinogalactans, rhamnogalacturons, riboflavin, β-carotene, phytostrols, citric acid, ascorbic acid, fruit acids, maleic acid, malic acid, hibiscic acid, oxalic acid, tartaric acid, (+)-allooxycitron acid-lactone, allohydroxycitric-acid, glycolic acid, utalonic acid, protocatechuic acid, cyanidin-3-glucoside, cyanidin-3-sambubioside, cyanidin-3-xyloglucoside, delphinidin, delphinidin-3-glucoside, delphinidin-3-sambubioside, delphinidin-3-xyloglucoside, delphinin, gossypetin, gossypetin-3-glucoside, hibiscetin, hibiscin, hibiscitrin, sabdaretin, sabdaritrin, fibre (crude), resin, fibre (dietery), minerals and ash.</td>
</tr>
<tr>
<td>Seed</td>
<td>Starch, cholesterol, cellulose, carbohydrates, campesterol, β-sitosterol, ergosterol, propionic acid, pentosans, pelargonic acid, palmitoleic acid, palmitic acid, oleic acid, myristic acid, methanol, malvalic acid, linoleic acid, sterculic acid, caprylic acid, formic acid, stearic acid, cis-12,13-epoxy-cis-9-octadecenoic acid, isopropyl alcohol, isomyl alcohol, ethanol, 3-methyl-1-butanol, fibre and minerals.</td>
</tr>
<tr>
<td>Leaf</td>
<td>α-Terpiny acetate, anisalddehyde, β-carotene, β-sitosterol, β-D-galactoside, β-sitosterol benzoate, niacin, fat, isomyl alcohol, is-2-propyl alcohol, methanol, 3-methyl-1-butanol, benzyl alcohol, ethanol, malic acid, fibre and ash.</td>
</tr>
<tr>
<td>Fruit</td>
<td>α-Terpiny acetate, pectin, anisalddehyde, ascorbic acid, calcium oxalate, caprylic acid, citric acid, acetic acid, ethanol, formic acid, pelargonic acid, propionic acid, isopropyl alcohol, methanol, benzyl alcohol, 3-methyl-1-butanol, benzaldehyde and minerals.</td>
</tr>
<tr>
<td>Root</td>
<td>Tartaric acid and saponin.</td>
</tr>
</tbody>
</table>

Data from Mahadevan et al. (2009)

Pharmacologically, infusions of the leaves are regarded as diuretic, cholerecic, febrifugal and hypotensive. Pharmacognosists in Senegal recommend roselle extract for lowering blood pressure. In experiments with domestic fowl, Roselle extract decreased the rate of absorption of alcohol and so lessened its effect on the system; the basis of its use as a remedy for after-effects of drunkenness (Morton, 1987). The calyx extract is used in the treatment of debility, hypertension, dyspepsia and heart ailments. The extracts of the leaves and flowers of Roselle are used internally as tonic tea for digestive and kidney functions (Saleh et al., 2013).

2.3. Biological activity and chemical composition of Hibiscus plataniolius L.

Saravanan et al. (2011) The biochemical parameters were increased in all diabetes rats; there parameters were decreased by the administration of ethanolic and aqueous hot extracts of leaves of H. plataniolius at dose of 100 mg and 150 mg/kg and are nearly similar to normal levels. H. plataniolius exhibited its scavenging effect in concentration dependent manner on hydrogen peroxide and property of metal chelating and reducing power. From this study it has been concluded that the ethanolic and aqueous hot extracts of leaves of H. plataniolius having good antioxidant, hypoglycemic and hypolipidemic effect.

2.5. Biological activity and chemical composition of Hibiscus mutabilis

The plant is having anti-inflammatory, antibacterial activity (Barve et al., 2010). The following chemical constituents were found in stem: naringenin-5,7-dimethyl ether, 4′-β-D-xlyopyranosyl-β-D-arabinopyranoside, and eriodictyol-5,7-dimethyl ether-4′-β-D-arabinopyranoside. The flowers showed to have: quercetin, quercemeritrine, quercetin-3-D-Xyloside, quercetin-3-sambubioside, isoquercetin, meratin, hybridin, kaempferol, hyperin, gualjaverin, Cyanidine-3-xlosyl glucose, cyanidin-3-monoguloside, hibiscones, hibiscocinones. Leaves reported to own: β-Sitosterol, β-Carotene, and Quercetin (Ishikura, 1973). It has previously reported that in addition to cyanidin 3-xyllosylglucose (ilicicyanin) and cyanidin 3-glucoside, the red petals of Hibiscus mutabilis f. versicolor contain the glycosides of quercetin and kaempferol (Ishikura, 1982). In the study of Ishikura (1973), five flavonol glycosides were isolated from the EtOAc extract. Glycosides were dark changing to yellow with NH3vapor. quercetin 3-sambubioside, isoquercitrin, hyperin , quercetin 3-a-L-arabopyranoside (gualjaverin) and avicularin.
The antioxidant activity of the extracts of seedcake has been extensively studied (Matthäus, 2002; Mariod et al., 2006). Recently, Mariod et al. (2012) reported that the Kenaf seedcake extract/fractions showed inhibitory activity of β-carotene bleaching and corn oil oxidation. Also, the extract/fractions were scavenged for the DPPH radical. The ethyl acetate fraction showed the highest DPPH radical scavenging activity. Therefore, the rich phenolic fractions of kenaf seedcake may represent a potential source of natural antioxidants. The antioxidant activity of the extracts from kenaf seedcake might also result from the presence of plant protein (33.0% protein content). Soluble proteins of legume seeds contain compounds of stronger antioxidant activity (Patel et al., 2001). Moreover, it has been shown that soluble proteins from plant seeds are capable of inhibiting lipid peroxidation in oil-in-water emulsions at pH 7.0 (Starzynska et al., 2008).

2.7. Biological activity and chemical composition of Hibiscus esculentus L.

The plant is originally an Indian plant it is now grown in many other areas of the world including the Middle East, Africa and the Southern States of the USA (Woolfe et al., 1977). The plant was also supposed to have come from Ethiopia from where it was propagated in North Africa, in Arabia and India, Egyptians being the first to plant it (Nzikou et al., 2006). A concentration of 200mg/L of crashed seeds water extracts was found effective to inhibit the growth of Salmonella, Shigella and Enterobacter (Nwaiwu et al., 2012).

2.8. Biological activity and chemical composition of Hibiscus tiliaceus L.

The extracts of H. tiliaceus were shown good antioxidant property (IC₅₀=86.5 μg/mL) and IC₅₀ of the sample (ascorbic acid) was 15.00 μg/mL. The ethanol extract of H. tiliaceus also showed activity against three strains of bacteria S. aureus (gram positive), E. coli and Salmonella paratyphi (Ramproshad et al., 2012). Leaves of H. tiliaceus had the strongest antityrosinase activity and have potentials to be developed into functional food and skin care products. Flowers of H. tiliaceus L. are widely used for birth control and for treating skin infections (Rosa et al., 2006).

Overall, the antimicrobial activity due to flavonoids may be because of their structure, as they have the ability to form a combined complex with bacterial cell walls (Cowan, 1999) Also, with the number of hydroxyl groups present on the phenolic ring there is increased hydroxylation, and with increased hydroxylation there will be increased antimicrobial activity. Cowan (1999) reported that the site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity. Flavonoids are also hydroxylated phenolic substances but occur as a C₆–C₃ unit linked to an aromatic ring. Because they are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms.

Plant containing quercetagettin-7-arabinosylgalactoside, a flavonoid has been used extensively to treat infectious disease (Tereschuk et al., 1997). Many other phytochemical preparations with high flavonoid content have also been reported to exhibit antibacterial activity (Salem et al., 2013). It has been reported that saponins and polyphenolic Compound have potent antimicrobial activity (El-Samawaty et al., 2013; Kamal et al., 2012; Ramalingam et al., 2009).

3. Conclusion

In this review article, the Hibiscus species showed significant antimicrobial and antioxidant properties against common human pathogens tested. Some of the bioactive constituents of these plants were isolated, purified and analyses for possible use in making drugs. Thus these plants have great medicinal potential for the therapy of infection.

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