



Preliminary Phytochemical Screening and Antioxidant Content of Leaves and Berries of Makoi

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Abstract

Makoi is well-known medicinal herb found in India that have bioactive components which attribute to a strong free radical scavenging activity and can modulate many diseases. In the view of the above research facts, the present investigation was undertaken to study the phytochemical screening (alkaloids, tannins, flavonoids, saponins, glycosides, phenolic compound, and phytosterols) and antioxidant potential (total phenolic content, total flavonoids content and tannins content) of both leaves and berries of Makoi plant. The study results revealed that Phytochemical screening of both leaves and berries showed the presence of all phytochemicals; alkaloids, tannins, flavonoids, saponins, glycosides, phenolic compound) except phytosterols. Whereas, antioxidant potential showed that leaves of Makoi had significantly higher content of total phenols ($12.16 \pm 0.05 \text{mgGAE/g}$), total flavonoids ($2.82 \pm 0.05 \text{mgQE/g}$) and tannins ($1.25 \pm 0.05 \text{mg/g}$) as compared to berries. Thus, the result of this study concluded that the leaves and berries of Makoi contain appreciable amount of antioxidants, hence can be beneficial to treat the various metabolic and degenerative diseases.

Keywords: Antioxidant Potential, Makoi, Phytochemical Screening

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Introduction

Free radicals are highly reactive molecules or chemical species capable of independent existence. The cytotoxic effect of free radicals is deleterious to mammalian cells and mediates the pathogenesis of many chronic diseases such as cardiovascular diseases, diabetes mellitus, cancer etc. Therefore, medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects and more than 80% of population of developing countries is dependent on traditional folk medicine therapies for treating their ailments. Most of the plants have protective biochemical functions of naturally occurring antioxidants in the cells (Patel *et al.*, 2009). Several pharmaceutically active

constituents of plants have been assessed to defend against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. Naturally occurring antioxidants in plant cells include peptide defence mechanisms which are catalases, peroxidases, superoxide dismutase, glutathione, proteins, tocopherols, flavonoids, phenolics, nitrogen compounds, carotenoids and chlorophyll derivatives (Kumar *et al.*, 2008).

The plant Makoi (*Solanum nigrum* Linn) is commonly known as black night shade in English, Kachchipandu in Telugu and Munatakali in Tamil, belongs to the *Solanaceae* family (Jimoh *et al.*, 2010). It is an annual weed that grows up to 60 cm tall, branched, erect, usually grows in moist habitats in different kinds of soil, including

dry, stony, shallow, or deep soils, crop fields. It can be cultivated in tropical and subtropical agro climatic regions. The stem may be smooth or bear small hairs (trichomes), the flowers usually white in colour, have five regular parts and are up to 0.8cm wide. The leaves are alternate, opaque, ovate, smooth, finely hairy, matt and dark in colour with irregularly toothed wavy margin. The berries are globular, dark green, matt berries 5-13 mm across, matt black when ripe, which contain many flattened finely pitted, yellow to dark brown woody seeds approximately 1.5 mm long.

It has an extensive range of medicinal value due to the presence of protein, vitamins and minerals and variety of natural bioactive compounds such as steroidal lactones, glycosides, alkaloids and flavonoids. Thus, it is commonly used in Ayurvedic medicine in India as tonic or supplement for the treatment and prevention of metabolic disorders. The herb also possesses antiseptic, antidysenteric and antidrug of cardiac, skin diseases, psoriasis and inflammation of kidney. The fruits are used as a tonic, laxative, appetite stimulant; and also for treating asthma and "excessive thirst" (Al-Daihan, 2008). The extracts of leaves are also used to alleviate liver-related ailments, including jaundice. It is also used in the Oriental systems of medicine for various purposes-as an antitumorogenic, antioxidant (Lee and Lim, 2003), anti-inflammatory (Zakaria *et al.*, 2006), hepatoprotective (Raju *et al.*, 2003) and antipyretic agent (Kaushik *et al.*, 2009). Hence, in the light of the above research facts, the present investigation was undertaken with the objective to study the preliminary phytochemical screening and antioxidant potential of leaves and berries of makoi.

Materials and methods

Sample collection and preparation of powder

The leaves and berries of makoi were collected from the Krishi Vigyan Kendra of

Bulandshahr, Uttar Pradesh. The leaves and berries were washed in tap water and shade dried after which they were reduced into fine powder by grinding and packed into air tight container for further analysis.

Preparation of aqueous extract of leaves and berries of Makoi

20g of powdered plant material was kept in 200ml conical flask and add 100ml of distilled water. The mouth of the conical flask was covered with the aluminium foil and kept in a reciprocating shaker for 25 minutes for continuous agitation at 150 rpm for thorough mixing. Then extracts were filtered by using muslin cloth followed by Whatman filter paper No. 42 (125mm). The contained was filtered by using rotator vacuum evaporator with the water bath temperature of 65°C and finally the residues were collected and used for the analysis (Nagappan, 2012).

Preliminary phytochemical screening

The filtrate of leaves and berries powder were tested for the presence of various bioactive compounds namely alkaloids (Mayer's reagents), tannins (Ferric chloride test), flavonoids (Shonoda test), saponins (Froth Test), glycosides (Legal's test), phenolic compound (Ferric Chloride) and phytosterols (Liebermann- Burchard's Test) (Oluduro, 2012).

Determination of antioxidants content

Total phenols content: Total phenols were determined by Folin-Ciocalteu reagent using gallic acid (GA) as the standard. The leaves and berries extract or processing water (50 µl), distilled water (3 ml), 250 µl of Folin-Ciocalteu reagent solution and 7% NaCO₃ (750 µl) were mixed in a tube and incubated for 8 min at the room temperature. A dose of 950 µl of distilled water was added. The mixture was allowed to stand for 2 hours at the room temperature. The absorbance was measured at 765nm against a reagent blank, which was composed of same reagents except that 50 µl of distilled water. The total phenolic content was expressed as gallic acid equivalents (mg

of GAE/g sample) through the calibration curve of gallic acid. Linearity range of calibration curve was 50-1000 μ g/ml ($r = 0.99$) (McDonald *et al.*, 2001).

Total flavonoids content: The total flavonoids were determined using a colorimetric method, as described by Shiva *et al.*, 2007. Briefly 0.1 ml of the methanolic extract was diluted with 0.9 ml of methanol. Aliquots of diluted extracts (0.5 ml) were added to test tubes and mixed with 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M aqueous potassium acetate and 4.3 ml of methanol. After standing for 40 min at room temperature, the absorbance of the reaction mixture was measured at 415 nm. Quercetin was used as a standard compound in the range of 50-200 μ g/ml concentration to construct a standard curve. The amount of total flavonoids was expressed as quercetin equivalent in mg/g of dried extract.

Tannins content: The tannin content of the leaves and berries was assessed using the Folin-Denis reagent (FDR) (Rahate *et al.*, 2013). A known quantity of finely ground sample of leaves and berries was extracted in 100 ml of distilled water by boiling gently for 30 min. the extract was centrifuged for 20 min and the supernatant was made to a known volume. An aliquot of 2-4 ml was pipette into 25ml volumetric flask and 0.5ml of FDR 1N solution and 1 ml of sodium carbonate solution was made up and allowed to stand for 1 hour. The turbidity was eliminated by filtering. The absorbance of color developed was measured at 710 nm. The concentration of tannic acid expressed as mg/g sample.

Statistical analysis:

The results obtained were expressed as Mean \pm SD and Paired 't'-Test of three determinations and also statistically analyzed to ascertain its significance. The significance was estimated at ($p \leq 0.05$ level).

Results and Discussion

Table 1 depicts that the phytochemical screening of both leaves and berries of Makoi.

Leaves and berries of Makoi contain all phytochemicals such as alkaloids, tannins, flavonoids, saponins, glycosides and phenolic compounds except phytosterols. The results in the study are in consonance with earlier report of Djaafar and Ridha, (2013) who noted the presence of alkaloids, tannins, flavonoids, saponins, glycosides and coumarines in both leaves and berries except terpenoids in leaves. Oyeyemi *et al.*, (2015) noted the presence of alkaloids, saponins, flavonoids, tannins, phenols, steroids, triterpenoids and the absence of glycosides in the berries of *Solanum anguivi*. It has been found that these phytochemicals act as an anti-inflammatory, anti-hypertensive, and anti-microbial. Alkaloids are plant-derived compound that is responsible to possess the antibacterial activity (Mantle *et al.*, 2000). Saponins and tannins also exhibit cytotoxic effects and growth inhibition making them suitable as tumor inhibiting agents (Akidanhunsu and Salowu, 2005). As a key source of flavonoids and phenolic compounds, leaves and berries of Makoi are proposed to have antioxidant properties. The apparent antioxidant and anti-mutagenic activities of the plant further suggest their potential usefulness in cancer transfer into cytosol by phenolic binding or insertion into the transporters of the outer membrane of the cell (Haskell *et al.*, 2004).

Table 2 depicts that the antioxidant activity of both leaves and berries of Makoi. The total phenols content (mgGAE/g) of leaves and berries were 12.16 ± 0.05 and 11.96 ± 0.05 respectively. This shows that leaves were significantly increased by 2.13% at $p \geq 0.05$ level when compared to berries. The values obtained compared favorably with leaves of *Solanum nigrum* (11.94mg/g) reported by Akuwugbo *et al.*, (2008). Likewise, the study reported by Govindan *et al.*, (2012) that berries of *Solanum muricatum* contain $(13.99 \pm 0.50$ mg GAE/g) of phenol which is agreement with the present study.

Table 1: Phytochemical screening of aqueous extracts of leaves and berries of Makoi

Phytochemicals	Reagents/Methods adopted	Leaves	Berries
Alkaloids	Mayer's reagents	+ve	+ve
Tannins	Ferric chloride test	+ve	+ve
Flavonoids	Shinoda test	+ve	+ve
Saponins	Froth test	+ve	+ve
Glycosides	Legal's test	+ve	+ve
Phenols	Ferric chloride	+ve	+ve
Phytosterols	Liebermann –Bur chard's Test	-ve	-ve

- Absence, + Present

Table 2: Antioxidant activity of leaves and berries of Makoi

Antioxidants	Leaves	Berries
Total phenols content (mg GAE/g)	12.16±0.05	11.96±0.05*(2.13%↓)
Total flavonoids content (mg QE/g)	2.82±0.05	1.62±0.01*(22.1%↓)
Tannins content (mg/g)	1.25±0.05	0.65±0.01*(48.0%↓)

Values are expressed as Mean ±SD *p≥0.05 (n=3) of triplicate determinations when compared with leaves and berries of Makoi

The estimated values for total flavonoids content (mgQE/g) of both leaves and berries were 2.82±0.05 and 1.62±0.01 respectively. This shows leaves were significantly increased by 22.1% at p≥0.05 level when compared with berries. The values obtained compared positively with leaves (2.31 ±0.47mg/g) and berries of *Solanum nigrum* (2.11±0.16 mg/g) reported by Gbadamosi and Afolayan, (2016). Whereas, the study reported by Alam *et al.*, (2012) that ethanol extract of *Solanum nigrum* berries contain 0.62mgQE/g of flavonoids which is lower than the data observed by present study.

The tannins content (mg/g) of leaves and berries were 1.25±0.05 and 0.65±0.01 respectively. This shows that leaves were significantly increased by 48.0% at p≥0.05 level when compared with berries. The values for tannins in this study were comparatively higher with leaves of *Solanum nigrum* (0.14±0.01mg/g) reported by Akuwugbo *et al.*, (2007) and with berries of *Soalnum anguivi* (0.17±0.07mg/g) reported by Oyeyemi *et al.*, (2015)

Conclusion

On the basis of present results it can be concluded that leaves and berries of Makoi bear potent antioxidant activity. The antioxidants act as defense mechanism that protects against oxidative damage, and include compounds to remove or repair damaged molecules and sufficient intake of antioxidants is supposed to protect against diseases. The phytochemical antioxidants have potent potential to neutralize free radicals or oxidants responsible for the cell damage. Thus, the present study scientifically validates and strengthens the candidature of Makoi in the preparation of medicinal aids to combat the wide spectrum of myriad diseases arising due to oxidative stress.

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Conflict of interest statement

We declared that we have no conflict of interest.

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