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PROXIMATE, PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY (IN VITRO AND EX VIVO) OF MORUS INDICA VARIETIES

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ABSTRACT: Plants rich in antioxidants and phytochemicals especially phenolic groups play a protective role in health and disease conditions, like cancer, diabetes, heart disease hypertension etc. by counteracting reactive oxygen species and inhibiting the lipid oxidation. *Morus indica* is non-toxic natural therapeutic plant used as herbal medicine as hypoglycemic, hypotensive, and diuretic properties. In the present study proximate, phytochemical and antioxidant profile in dehydrated leaves powder of *Morus indica* varieties, M5, V1 and S36 were estimated. The 100% methanol, 80% methanol (methanol & water, 8: 2), dechlorophyllised, aqueous cold and hot extracts were prepared and their polyphenol content, antioxidant activity of inhibiting oxidation in oil emulsion and liver microsomes was evaluated at 300 to 500µg. There was no significant ($P<0.05$) difference in the moisture, fat and ash content. All the three samples were found to be good sources of major minerals viz. Iron, Calcium phosphorus and also of trace elements like manganese, zinc, copper, potassium. S36 found to be rich in phytochemicals and antioxidants. The polyphenol content was in the order of The polyphenol content of the extracts was in the order of dechlorophyllised>Methanol> 80% methanol > Aq. c old and hot. In oil emulsion, no significant difference in inhibiting oxidation was observed in methanol and 80% methanol extracts, whereas in dechlorophyllised, aq. cold and hot extracts V1 and S36 showed higher activity. In microsomes, S36 variety was more potent in inhibiting oxidation in all extracts except Aq. cold. S36 was found to be good source of phytochemicals and showed more potency in inhibiting the oxidation in two food and biological systems.

INTRODUCTION: Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects such as phytochemicals and antioxidants^{1, 2}. The term Phytochemicals is usually used to refer compounds found in plants that are not normally involved in primarily metabolic process such as photosynthesis and cell respiration of plants. The biological activities elicited by a plant are primarily due to the presence of these chemical constituents in the plant.

Currently much research is focused on the beneficial effects of bioactive phytochemicals present in micro level in our daily diet³. Several epidemiological studies suggest that plants rich in antioxidants play a protective role in health and diseased condition and their consumption lower risk of cancer, heart disease, hypertension and stroke. The major groups of phytochemicals that may contribute to the total antioxidant capacity of plant include polyphenols and vitamins (C and E)⁴.

With the focus being shifted towards finding alternatives for synthetic food ingredients, natural substances having antioxidant properties need to be further explored⁵. Many plant species have been investigated in search of novel antioxidants and there is still demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards the identification of plant materials, rich in antioxidants⁶.

However, besides the role played in human and animal nutrition, knowledge of proximate, phytochemical and micronutrient composition is fundamental for understanding modes of action of medicinal plants in general. It is the diverse composition of these components in plants that places them at advantage position over and above chemotherapy in management of complex diseases such as diabetes mellitus⁷. Each medicinal plant species has its own nutrient composition besides having pharmacologically important photochemicals. These nutrients are essential for the physiological functions of human body. Such nutrients and biochemicals like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes^{8,9,10}.

Morus indica (Mulberry tree) of the family Moraceae has been widely cultivated in countries all over the world including temperate to tropical areas. Different parts of the plant are used as herbal medicine for blood serum glucose reduction, cholesterol and lipids levels reduction, antiphlogistic, diuretic and expectorant effects. It occupies an important position in the holistic system of Indian medicine 'Ayurveda' which has its roots in antiquity and has been practiced for centuries. The leaves of mulberry are nutritious, palatable, nontoxic and also enriched with different active principles¹¹.

The antihyperglycemic effects (properties) of mulberry leaves were investigated in streptozotocin induced diabetic rats^{12,13}. There are few reports on the antioxidant activity of *Morus indica*¹⁴ but the complete information on proximate, phytochemical and antioxidant composition of the plant is scarcely reported.

In the present study, three different varieties of *Morus indica* (L), M5, V1 and S36 were studied for their proximate, antioxidant and phytochemical composition.

MATERIALS AND METHODS:

Chemicals: Tocopherol, β carotene, 1,1, Diphenyl – 2- picryl hydrazyl (DPPH), 5-Dithio (bis) nitro benzoic acid (DTNB), were purchased from Sigma Aldrich, Bangalore, India. All other chemicals and reagents used in the study were of analytical grade.

Plant material: The leaves of three varieties of *Morus indica* (M5, V1 and S36) were collected from Central Sericulture Research and Training Institute (CSRTI), Mysore. The samples were identified by Dr. Shivamurthy, Department of studies in Botany, University of Mysore and voucher specimen was retained in the laboratory for future reference. The leaves were washed, dried in the oven overnight at 50°C, powdered, passed through 60 mesh and stored at 4°C till further use.

Proximate composition (dry basis): Moisture content was determined by using Moisture analyser (Metler Toledo MJ33, Lab systems Bangalore, India). Fat, protein, ash, total fiber (soluble and insoluble fiber) was estimated as per the AOAC standard methods¹⁵, total starch¹⁶, total sugars and free glucose were estimated by Dubois *et al.*,¹⁷ method. Iron, Calcium, Phosphorus, Copper, Manganese, Zinc, Sodium, Potassium, Magnesium and chromium were analyzed using Atomic Absorption spectroscopy (ELICO SL-168, range 160-900nm).

Phytochemical composition (dry basis): Total phenols were extracted from a weighed portion (50-500 mg) of dried sample with 5ml of 1.2M HCl in 50% aqueous methanol for 2 h and analyzed by Folin-Ciocalteu micro method. Results are expressed as μmol Gallic acid equivalent g^{-1} dry weight¹⁸. Flavonoid content was determined by a pharmacopoeia method using Rutin as a reference compound¹⁹, alkaloids by the method based on the reaction with bromocresol green (BCG) using Atropine as a standard²⁰ saponins by using vanillin/sulfuric acid reagents and Diosgenin as standard²¹, tannins by the method of Makkar *et al.*,²² were estimated.

Antioxidant profile: In dehydrated sample, different antioxidants components were estimated by using standard methods. α - Tocopherol was extracted by direct saponification of dried sample and estimated based on formation of a red complex from reaction of α, α' -bipyridyl with ferrous ion due to reduction of ferric ion by tocopherol²³. β - Carotene was quantified by column chromatography, followed by measuring the absorbance of elute at 450 nm against standard β -carotene²⁴. Reduced glutathione was determined based on the development of a yellow compound due to reaction of 5,5-Dithio (bis) nitro benzoic acid (DTNB) with compounds containing sulphhydryl groups²⁵.

Preparation of solvent extracts: A 15g dehydrated sample of all the three varieties individually was extracted with 100 ml solvent [100% methanol, methanol and water (80:20) and 100% water] for 6 h in a mechanical shaker. The extracts were filtered and filtrates were evaporated at 40° C under reduced pressure to dryness in a rotary evaporator (Superfit, India). The residue of each extract was stored in airtight container at 4°C until further use. In case of hot water extract, the sample was added to boiling water and extracted for 15 min and filtered. The filtrate of both cold and hot water extracts was freeze dried and stored at 4°C until further use.

Dechlorophyllised extract: Since the samples were rich in chlorophyll, an extract was prepared by separating the chlorophyll by the method described by Rich et al. Briefly, hexane was added to the 80% methanol extract and shaken for 30 minutes, as chlorophyll is readily soluble in hexane, a chlorophyll rich hexane layer is formed on the top of the extract leaving the other polar components especially phenolic compounds in the water and methanol layer and this polyphenol (30-40%) rich extract was further dried and stored in air-tight container at 0°C until used. The Total polyphenol content of all the extracts was estimated¹⁸.

Antioxidant activity:

Inhibition of Lipid Oxidation: The antioxidant activity of the above extracts (300-500 μ g) was determined in an edible oil emulsion and liver microsomes by modified method of TBARS method^{26, 27}.

Antioxidant activity in Lipids: Five grams of oil (sunflower) was weighed and an emulsion was prepared in phosphate buffer of 0.01M, 7 pH, with few drops of tween 20 and the volume was made upto 10ml. To different concentrations (300-500 μ g) of extracts, 300 μ l of emulsion, 450 μ l of fentons reagent was added and volume was made upto 2ml and incubated at 50°C for 6hrs. A control was run without extract. After incubation, 1ml of thiobarbituric acid (TBA) was added and heated in boiling water bath for 30min. and cooled immediately. The inhibition of lipid peroxidation in sun flower oil was determined by TBA, in which the secondary oxidation products (TBARS, expressed as MDA equivalents) formed by oxidation of oil was determined by measuring the absorbance at 532 nm.

Preparation of Microsomes: A healthy male adult rat was sacrificed to get fresh liver. The procedure followed for microsomes preparation was as reported by Shapiro & Rodwell²⁸. The liver was immediately removed and placed in cold buffer (0-4° C) at pH 7.4. The buffer contained 0.1 M triethanolamine.HCl, 0.02 M EDTA, and 2.0mM dithiothreitol. The liver tissue was minced with scissors and homogenized with six strokes of a smooth-walled, glass Remi homogenizer and centrifuged for 10 min at 12,000 g to remove cell debris and mitochondria.

After the supernatant solution was carefully removed to prevent contamination with mitochondria and recentrifuged for 10 min at 12,000 g to ensure removal of mitochondria, it was then centrifuged at 60,000 g for 60 min. The 60,000g microsomal pellet was then rinsed with buffer and frozen in a freezer (-20°C). Frozen microsomes were resuspended in 0.1 M triethanolamine buffer, pH 7.4, and containing 0.02M EDTA and 10mM dithiothreitol and allowed to stand 60 min packed in ice. The resuspended microsomes to be used for the assay were diluted with buffer to give a protein concentration of 5-10 mg/ml.

Antioxidant activity in Microsomes: Here in place of oil emulsion, liver microsomes of 1mg protein concentration was taken, 300-500 μ g extracts with Fentons reagent and incubated at 50°C for 2 hrs. After incubation 1ml TCA 10% and 1ml TBA was added and heated in boiling water bath for 15min. and cooled in ice bath immediately. After cooling 2ml of butanol was added and pink color was read at 532nm. A control was run without samples.

Statistical analysis: Results were expressed as the mean \pm standard deviation (SD). SPSS (version 16.0; SPSS Inc. Chicago, IL, 2007) statistical program was used for data analysis.

RESULTS: The proximate composition of the *Morus indica* varieties is given in **table 1**. No significant ($P < 0.05$) difference was observed between moisture and ash content of the three varieties, and the protein content of the M5 variety was significantly ($p < 0.05$) higher (14.17 ± 0.63^a) than the other two varieties, (V1- 11.58 ± 1.22^b ; S36- 12.15 ± 0.304^b). The fat content of the V1 (7.9 ± 1.68^b) was higher than M5 (5.09 ± 0.11^a) and S36 (6.45 ± 2.01^{ab}). The mineral composition of M5 was found to be rich in calcium (682.76 ± 3.06 mg), phosphorus (164 ± 11 mg), sodium (0.014mg) and magnesium (3.91 mg). Manganese, copper and potassium were present at high percent in V1 and

S36 was rich in Iron and zinc. The carbohydrate profile is given in table 1. The V1 and S36 were found to be significantly high ($p > 0.05$) in carbohydrates than the M5. The total fiber content was significantly high in V1 and in case of S36 total sugars and free glucose was significantly high. The antioxidant and phytochemical composition is given in **table 2**.

Among three varieties S36 was found to be rich in maximum phytochemical and antioxidant components (glutathione- 788mMoles, β carotene-87.30m), flavonoids-1.15mg/g extract, , alkaloids- 100.66mg/g and tannins-1546.67mg) than other two varieties. M5 was rich in α tocopherol-34 mg, saponins-135.3 mg and steroidal saponins-39.63 mg and V1 was rich in polyphenols -1.406g. The polyphenol content of the extracts was in the order of dechlorophyllised >Methanol> 80% methanol > Aq. c old and hot (table2)

TABLE 1: PROXIMATE COMPOSITION OF THE DIFFERENT VARIETIES OF MORUS INDICA

Components	M5	V1	S36
	g/100g (dry basis)		
Moisture	8.71 ± 0.20^a	8.67 ± 0.12^a	8.52 ± 0.22^a
Protein	14.17 ± 0.63^a	11.58 ± 1.22^b	12.15 ± 0.304^b
Fat	5.09 ± 0.11^a	7.9 ± 1.68^b	6.45 ± 2.01^{ab}
Total fiber	10.86 ± 1.00^a	12.81 ± 0.91^b	9.78 ± 0.93^a
Ash	10.06 ± 0.78^a	10.93 ± 0.70^a	10.69 ± 1.26^a
Iron*	5.80 ± 0.28	5.86 ± 0.28	8.39 ± 0.48
Calcium*	682.76 ± 3.06	626.183 ± 8.35	650.32 ± 1.69
Phosphorus*	164 ± 11	136.33 ± 3.50	152.30 ± 3.05
Sodium*	0.014	0.010	0.009
Manganese*	2.4	5.83	4.43
Zinc*	0.86	0.90	1.03
Copper*	0.416	0.483	0.366
Potassium*	0.9	1.05	0.92
Magnesium*	3.905	2.223	2.172
Chromium*	ND	ND	ND
Soluble fiber	5.63 ± 0.60^a	6.77 ± 0.26^b	6.58 ± 0.53^{ab}
Insoluble fiber	5.23 ± 0.40^a	6.04 ± 0.65^a	3.2 ± 0.40^b
Total sugars	0.92 ± 0.017^a	1.01 ± 0.147^{ab}	1.26 ± 0.079^b
Total glucose	18.10 ± 2.57^a	20.10 ± 2.67^a	20.58 ± 3.79^a
Free Glucose	0.162 ± 0.053^a	0.250 ± 0.046^{ab}	0.292 ± 0.012^b
Total carbohydrate	30.042 ± 3.64	34.17 ± 3.68	31.912 ± 5.741

*mg/100g dry basis

TABLE 2: PHYTOCHEMICAL PROFILE IN DIFFERENT VARIETIES OF MORUS INDICA

Phytochemical	M5	V1	S36
	/100g (dry basis)		
α -Tocopherol (mg)	34 \pm 3.05 ^a	24 \pm 2 ^b	28 \pm 2 ^b
Glutathione (m Moles)	520 \pm 0.00 ^a	550 \pm 4.642 ^a	788 \pm 15.17 ^b
β carotene (mg)	69.35 \pm 1.64 ^a	73.50 \pm 4.77 ^a	87.30 \pm 3.12 ^b
Polyphenols (mg)	0.505 \pm 0.02 ^a	1.406 \pm 0.155 ^b	0.622 \pm 0.023 ^c
Flavonoid (mg/g)	0.783 \pm 0.01 ^a	1.05 \pm 0.07 ^b	1.15 \pm 0.01 ^b
Alkaloids* (mg)	46.83 \pm 0.288 ^a	72.5 \pm 6.06 ^b	100.66 \pm 9.51 ^c
Saponins* (mg)	136.33 \pm 8.96 ^a	103.33 \pm 0.577 ^b	114.66 \pm 11.66 ^b
Steroidal saponins* (mg)	39.96. \pm 6.01 ^a	28.3 \pm 3.3 ^b	22.96 \pm 3.35 ^b
Tannins (mg)	873.33 \pm 20.81 ^a	1003.33 \pm 50.33 ^b	1546.67 \pm 55.07 ^c
Polyphenol content			
Meoh	1.16	2.33	1.68
80% Meoh	5.3	2.8	5.3
Dc	5.6	3.7	5
AC	1	1.50	1.80
AH	1.5	1.91	2.16

*-expressed /g extract (p<0.05)

The inhibition of oxidation in oil emulsion and microsomes was measured at different concentrations (300-500 μ g) and IC₅₀ values of the different solvent extracts is given in **figure 1 and 2**. Among different extracts methanol and 80% methanol was more effective in inhibiting the oxidation rate followed by dechlorophyllised, aqueous cold and hot extracts.

In all the samples except Aq. cold extract, S36 exhibited maximum activity than V1 and M5 in a dose dependent manner. There was no significant difference between the methanol and 80% methanol extracts in inhibiting the oxidation in oil emulsion whereas in dechlorophyllised, aq. cold and hot extracts of V1 and S36 showed significantly (p<0.05) higher inhibition. In microsomes, the IC₅₀ value of S36 was between 170-210 μ g. in all extracts except Aq cold, 647 μ g. The IC₅₀ value of V1 was less incase of Aq cold extract.

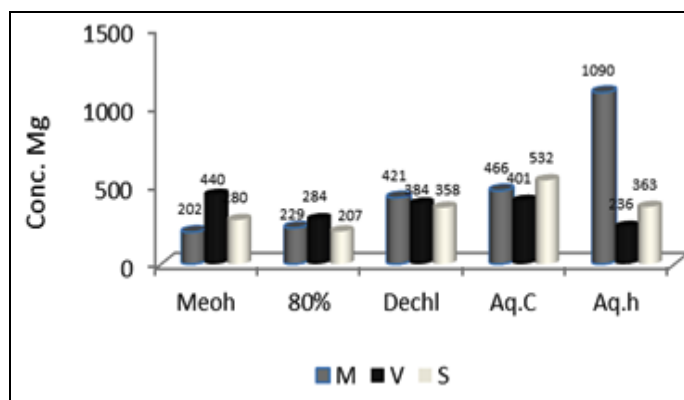


FIGURE 1: IC₅₀ OF THE MI EXTRACTS IN INHIBITING THE OXIDATION IN OIL EMULSION

(Meoh- Methanol; 80%- Methanol-8: water-2; Dechl. - Dechlorophyllised; Aq.C- Aqueous cold; Aq.H- Aqueous hot) Values on the bars refer to concentration of extract in μ g

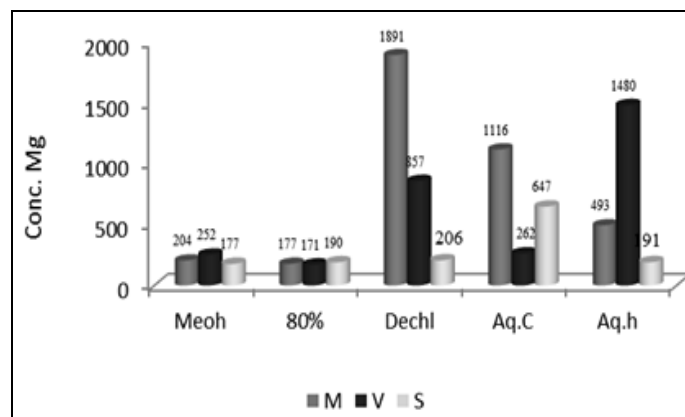


FIGURE 2: IC₅₀ OF THE MI EXTRACTS IN INHIBITING THE OXIDATION IN LIVER MICROSOMES.

(Meoh- Methanol; 80%- Methanol-8: water-2; Dechl. - Dechlorophyllised; Aq.C- Aqueous cold; Aq.H- Aqueous hot), Values on the bars refer to concentration of extract in μ g

DISCUSSION: Many traditional plants remedies are known in folk medicine and used for treatment and management of diabetes mellitus²⁹ and some have been validated by scientific studies to actually exert biological action against diabetes or its complications.

The medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as a dietary supplement to existing therapies and about 400 of such traditional plant remedy have been reported^{30, 31}.

The medicinal properties of these plants have been attributed to the biochemicals resident in the plant materials. Although studies have reported MI to exert an excellent antidiabetic effect^{13, 32}, the chemical composition of MI has been poorly investigated and most of the available information only deals with traditional and medicinal aspects. The aim of the present study was to determine the phytochemical and proximate composition of three varieties of *Morus indica* (M5, V1 and S36) leaves.

Hence, in the present study the proximate, micronutrient, carbohydrate and phytochemical compositions of 3 *Morus indica* (M5, V1 and S36) varieties were determined. In addition to their role in human and animal nutrition, knowledge of proximate, micronutrients and phytochemical composition is fundamental to the understanding of modes and mechanisms of action of medicinal plants in general. There are reports on the proximate, phytochemical and micronutrient composition of some medicinal plants which have been proved in diabetes therapy³³.

The results obtained from proximate analysis of leaves in all three varieties of MI reveals that the samples are good sources of carbohydrate and minerals with low fat content. This trend supports the reports on some medicinal plants with antidiabetic effect. The crude protein content shows they are fair sources and relatively less than some of the medicinal plants³⁴. Dietary fiber has positively been implicated in management of diabetes and post-prandial hyperglycemia. It delays gastric emptying or increase viscosity of GIT content thereby suppressing digestion and carbohydrate absorption. This mechanism is selectively advantageous in the threat or risk of hypoglycaemia, hyperinsulinemia and undue weight gain is absolved. Many of the Clinical trials (60–70%) have reported the association of high intake of soluble fiber with significant decreases in total and LDL cholesterol³⁵.

The mineral profile of the MI varieties shows all the three as good sources of iron, calcium and phosphorus. Minerals are naturally occurring chemical elements the body uses, to help and perform certain chemical reactions. Minerals form an integral part of functionally important organic compounds such as iron (Fe) in hemoglobin and Cytochrome or zinc (Zn) in insulin^{36, 37}.

Mineral deficiencies have manifested in forms of different disease conditions as goiter, rickets, and one form of metabolic dysfunction or the other. These K, Ca, Na and Mg mineral elements are very important in human nutrition. Calcium, potassium and magnesium are required for repair of worn out cells, strong bones and teeth in humans, building of red blood cells and for body mechanisms³⁸.

Concentrations of some of these macro elements obtained in MI varieties are appreciable (table 2). The biological roles for K and Ca are essential for disease prevention and control and may, therefore, contribute to some of the traditional medicinal influences of the plants. Trace elements such as manganese, iron and zinc are essential in enzymes metabolism. The concentrations of these elements in the plants are quiet important. Manganese is an important modulator of cells functions and play vital role in the control of diabetes mellitus. The importance of iron in maintaining the good health has been recognized³⁹.

Phytochemicals composition of the samples is given in table 2. As mentioned earlier S36 variety was found to be good source of many phytochemicals. Among the polyphenols all the varieties have shown some quantity of phenolics which are well known for their antioxidant activity, and also anti-microbial agents. Several reports indicate the antioxidant potential of medicinal plants may be related to the concentration of their phenolic compounds which include phenolic acids, flavonoids, anthocyanins and tannins⁴⁰.

Studies from our laboratory have reported a positive correlation between the polyphenols and antioxidant activity of *Moringa oliefera*, *Psidium guajawa*, *Aegle marmelos*^{41, 5}. Apart from using as antioxidants, Phenols and phenolic compounds are greatly used in skin infections and other wounds treatment and also for healing, when compared to other bactericides⁴². Phenolic compounds have an electron donor capability, moreover, due to this ability; phenolic compounds are readily oxidized to form phenolate ion or quinone, which is an electron acceptor. Phenols and phenolic compounds modify the prostaglandin pathways and due to this action, they prevent platelets from clumping and have the ability to block specific enzymes that cause inflammation; antioxidant, immune enhancers, anti-clotting and hormone modulators.

Flavonoids are water soluble phytochemicals and are hydroxylated phenolic substances. They show antioxidant activities and prevent oxidative cell damage and carcinogenesis. They have anti-cancer, anti-inflammatory activities and a large effect in lower intestinal tract and heart disease⁴³. Studies have reported the therapeutic effect of flavonoids against wide array of microorganisms, by making a complex with extracellular and soluble proteins and to complex with the bacterial cell wall. Flavonoids and other phenolic compounds are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anti-cancer activity⁴².

The biological function of alkaloids and their derivatives are very important in medicine and are used in analgesic, antispasmodic and bactericidal activities. However, alkaloids are mainly observed in large amount in flowering plants and they have an important physiological effect on mankind⁴⁴. They have the property of binding with cholesterol, bitterness and hemolytic activity in aqueous solution⁴⁵. In the present study, V1 and S36 variety were found to be good sources of flavonoids, polyphenols and alkaloids. Hence, MI varieties can be used against some degenerative diseases such as diabetes mellitus, cancer, etc.

Saponins comprise a large family of structurally related compounds containing a steroid or triterpenoid aglycone (sapogenin) linked to one or more oligosaccharide moieties by glycosidic linkage. In MI varieties saponins were found in appreciable amount. The primary biological effect of saponins is the interactions with cellular and membrane components. For example, saponins hemolyze red blood cells by nonspecific interactions with membrane proteins, phospholipids, and cholesterol of erythrocytes. Saponins are characterized by their hemolytic activity and foaming properties. Few studies have reported the use of saponin extract from medicinal plants in precipitation and coagulation of RBC and wound healing property⁴². Saponins are reported to affect the permeability of the small intestinal mucosal cells and thus have effect on active nutrient transport.

On the other hand, positive nutritional effects of specific saponins such as hypocholesterolemic effects and improvement of growth in various animal species have also been reported⁴⁴.

These medicinal properties bestow high medicinal activities of MI extracts varieties. Glutathione, tocopherol, β carotenoids and flavonoids etc. comes under second line defence antioxidants. β carotene is an excellent scavenger of singlet oxygen. Vitamin C directly interacts with radicals like $O_2^{\cdot-}$, OH. Glutathione is a good scavenger of many free radicals like $O_2^{\cdot-}$, HO and various lipid hydroperoxides and may help to detoxify many inhaled oxidizing all pollutants like ozone, NO_2 and free radicals in cigarette smoke in respiratory tract. Vitamin E scavenges peroxy radicals intermediates in lipid peroxidation and is responsible for protect PUFA present in cell membrane and LDL, against lipid peroxidation⁴⁵.

Lipid peroxidation is a process in which PUFA undergo oxidative damage resulting in lipid derived radicals such as alkoxy and peroxy radicals. In biological systems, antioxidants are capable of stabilizing or deactivating free radicals before they attack cells. The polarity of phytochemicals plays a key role in exhibiting antioxidant role at lipid phase especially at unsaturation site, which influence the chain breaking reaction⁴⁶.

Similarly in methanol and 80% methanol extracts, the antioxidants with high partition coefficient may be distributed hydrophobic compartments for the protection of lipids. In the present study, S36 found to be rich source of maximum phytochemicals. As mentioned earlier, a positive correlation exists between polyphenols and antioxidant activity⁴¹ and the polyphenol content of the extracts was in the order of dechlorophyllised > Methanol > 80% methanol > Aq. cold and hot.

In contrast, the antioxidant activity was maximum in methanol and 80% methanol, which may be due to the synergistic action of polyphenols and other phytochemicals. Dechlorophyllisation might have resulted in the removal of carotene and tocopherol along with chlorophyll which may be the reason for lower ability to inhibit oxidation in the two systems studied. Use of two lipid systems was helpful in studying the inhibition of oxidation by different extracts.

CONCLUSION: Varied proximate and phytochemical composition was observed in the three *Morus indica* varieties studied. Different extracts of MI exhibited protection against lipid peroxidation

indicating its role in prevention of oxidative stress. Further studies on characterization and isolation of the phytochemicals are underway. *Morus indica* is well known for its medicinal properties, present information will help in further exploring the sample as nutraceutical and also to study its therapeutic effect against degenerative diseases (diabetes mellitus, cancer), anti-inflammatory, lipid lowering effect especially in cholesterol metabolism. The high fiber content of *Morus indica* provides scope to be used as a functional ingredient in disease specific food formulations.

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