IJCRR
Vol 06 issue 09
Section: Healthcare
Category: Review
Received on: 15/03/14
Revised on: 07/04/14
Accepted on: 29/04/14

BIOACTIVE COMPONENTS OF SPINACH AND THEIR EFFECT ON SOME PATHO PHYSIOLOGICAL CONDITIONS: A REVIEW

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ABSTRACT

Spinach is a commonly consumed leafy vegetable packed with micronutrients and phytochemicals. It has functional ingredients such as lutein, betaine, flavonoids, neoxanthin, galactolipids. Each of these has its own physiological significance. Studies have indicated that regular consumption of spinach substantially lowers the risk of age related macular degeneration, one of the leading causes of irreversible blindness among adults. There is an inverse association between spinach consumption and cataract risk. Spinach leaf protein concentrate has a strong cholesterol lowering effect in rats. Age related changes on brain function can be delayed by long term consumption of spinach. It has high anti proliferative activity on cancer cells. However, people prone to oxalic acid stones are to reduce consumption of spinach. So quantityand frequency of spinach consumption can be increased.

Keywords:functional ingredients,antioxidant activity; age related macular degeneration; aging; cancer

INTRODUCTION

Spinach (Spinaciaoleracea) is a commonly consumed green leafy vegetable.Although it is native to central and southwestern Asia, now it is produced andconsumed throughout the world. Green leafy vegetables such as spinach form animportant part of a balanced diet. Their micronutrient content, along with acombination of low calorie content, low glycemic index, dietary fiber make it anappealing healthy choice.The contribution of dark green leafy vegetables to total micro nutrient intake of 2-5 year old children in rural South Africa is quite significant. For children consumingdark green leafy vegetables, imifina (collective term for various dark green leavesthat are eaten as a vegetable) and Spinach contributed significantly to the dietaryintake of calcium (21 to 39%), iron (19-39%), vitamin A (42 to 68%) and riboflavin (9 to 22%). This contribution can be increased if these vegetables are consumed morefrequently and by a larger proportion of children[1].

COMPOSITION OF SPINACH

Spinach is a rich and affordable source of micronutrients and phytochemicals. Itcontains vitamin C (28.1 mg), vitamin K(482.9), folate (194 mcg), calcium (99 mg), iron (2.71mg), magnesium, manganese, zinc etc per 100g of edible portion[2]. Xanthophyll is a class of carotenoids with oxygenated carotenes. Themolecular formula of xanthophyll is $C_{40}H_{56}O_2$. They are typical yellow pigments ofleaves. Spinach contains a variety of xanthophylls. Rangaswamy L et al. (2005)[3]have determined these by HPLC method. Table. 1 gives the xanthyphyll compositionof spinach.

- Bioactive Components

Studies indicate that spinach is a source of bioactive components such as lutein, betaine, flavonoids, neoxanthins and galactolipids. Table 2 gives the quantity of each of these components.

The general structure, physiological role and nutritional significance of each of these is discussed below.

Lutein: It is one of the natural carotenoids synthesized only by plants. It has thefollowing structure (Fig 1).

Lutein and zeaxanthin are the only carotenoids found in the retina. They are mainlypresent in the macula where they act as absorbers of blue light. By preventing asubstantial amount of blue light from reaching the underlying structures, they protectagainst oxidative damage induced by light. This oxidative damage is believed to playa role in the causation of age related macular degeneration (AMD).

Betaine: Betaine is a chemical compound alternatively called trimethylglycine, glycine betaine, lycine, and oxyneurine. It is a zwitterionic quaternary ammoniumcompound. The chemical structure is shown below (Fig 2). Betaine performs the following physiological functions. (Stuart A.S. C. 2004).[4]

1. It is an osmolyte. So it protects the cells, proteins and enzymes fromenvironmental stress such as lack of water, unfavourable temperature, orsalinity
2. It is a source of methyl groups. Therefore it participates in methionine cycle inkidney and liver.
3. Betaine can reduce the serum homocysteine concentration in case of mild orsevere hyper homocystinuria Via Methionine cycle.
4. It may have a role in epigenetics and athletic performance.

Deficiency of methyl groups results in hypomethylation which hasthe following consequences.Increase in plasma homocysteine concentration, decrease in s-denosyl methionine concentration, impaired hepatic fat metabolism resulting in fat accumulation (steatosis). This leads to dyslipedemia. Coronary, cerebral, hepatic and vascular diseases may result due to faulty liver metabolism.

Spinach is one of the food items with high betaine content (600-645 mg/100g).[5]: Betaine-aldehyde dehydrogenase was purified from spinachleaves and characterized by Keita A *et al* (1987).[6]

Flavonoids: They are a class of secondary plant metabolites widely distributed inplants. They function as plant pigments and are responsible for yellow / red / bluecolor. In vitro studies have shown that some of these flavonoids show anti-allergic, anti-inflammatory, anti-oxidant, anti-microbial and anti-cancer activities. There is verylimited in vivo or clinical research to prove or disprove this claim. Rudolf Edenharder *et al*[7] isolated 13 flavonoid compounds from spinach and some of these were foundto be potent antimutagens.

Neoxanthin: It is a type of xanthophyll found in green leafy vegetables such asspinach (Table 1). It is an intermediate in the biosynthesis of plant hormone abscisicacid and is synthesized from violaxanthin. It has the following structure (fig 3). A study by Luca Dall'Osto *et al* (2007)[8] has shown that neoxanthin acts as anantioxidant within the photosystem II super complex and neoxanthin protectsmembrane lipids from reactive oxygen species and superoxide anions. Eiichi Kotake-Nara *et al* (2001)[9] have shown that neoxanthin can reduce the risk of prostatecancer and the details are discussed under spinach and cancer.

Galactolipids: They are glycolipids with galactose as sugar group widely found inplant kingdom. They are present in cell membrane lipids.Monogalactosyldiacylglycerol (MGDG) and Diagalactosyldiacylglycerol (DGDG) arepresent in higher amounts in chloroplast membranes. They are believed to play arole in photosynthesis. The chemical structure of a common galactolipid is shownbelow (Fig 4).

Wang R (2002)[10] has estimated that spinach contains 3300-3880 (mg/kg) of MGDG (Monogalactosyldiacylglycerol), a galactolipid. Lars P. Christensen (2009)[11] hasreported that different studies have estimated MGDG (mg/kg) content of spinach onfresh weight basis as 546, 850 and 3300-38, 800 and DGDG (mg/kg) content of spinach on fresh weight basis as 563.

A study has shown that extraction of dry spinach leaves resulted in a glycolipid enriched fraction. This contained 3 types of glycolipids-MGDGs, DGDGs and sulphoquinovosyldiacyl glycerol at a high ratio. These play a role in DNA synthatase inhibitory activity, inhibition of cancer cell growth and antitumor activity (Mizushima *et al* 2005).[12]

Bioavailability of Nutrients

Bioavailability, defined as the proportion of an ingested trace element in food that isabsorbed and utilized for normal metabolic and physiological functions or storage (Jackson, 1997)[13], is an important factor in deciding the nutritional status. The chemical form in which a nutrient is present in food, nutrient interactions within the food, the presence of anti nutritional factors and processing of food all influence the bioavailability of a nutrient. Spinach contains anti nutritional factors like oxalic acidwhich reduce the absorption of calcium and iron by forming insoluble complexes withthem. Oxalic acid may lead to oxalate stones in the urinary tract of some people.Therefore people who have oxalate stones in their urinary tract are advised toreduce eating vegetables like spinach. Dietary fiber may also interfere withabsorption of nutrients.

Studies have shown that bioavailability of nutrients in spinach depends on the formin which it is consumed. β carotene present in raw spinach has less bioavailabilitycompared to cooked and pureed spinach (Cherry Rock *et al* (1998).[13] The plasma βcarotene levels were three times higher after daily consumption of carrots andspinach for 4 weeks in processed form than when they were consumed in raw form.Bioavailability of β carotene is increased by the disruption of cell wall structure andloss of cellular integrity of spinach leaves (Jacqueline *et al*).[14] The table 3 showsbioavailability of spinach in different forms. Bioavailability of lutein is higher than that of beta carotene.

Antioxidant Constituents:

Different antioxidant constituents present in a vegetable such as alpha tocopherol, beta carotene, vitamin C, selenium or phenolic compounds etc. contribute to the totalantioxidant capacity of a vegetable. Amin Ismail *et al* (2004) 15 have shown that thehigh antioxidant activity of spinach is due to high alpha tocopherol, beta caroteneand ferulic acid. The total antioxidant activity of spinach by different methods is given in Table 4.

The active fractions from aqueous spinach extracts have been chemically identified (Margalit Bergman et al (2001).[16] There are 4 hydrophobic fractions (glucuronic acidderivatives of flavonoids), 3 fractions of trans and cis isomers of p-coumaric acidderivatives and others are meso-tartarate derivatives of p-coumaric acid.

There is a variation in the flavonoid content among different genotypes of spinach (Mi Jin Cho *et al* (2008).[17] 18 flavonoids representing glucuronides and acylated diand triglycosides of methylated and methylene dioxide derivatives of 6 oxygenatedflavonols were identified (patuletin, spinacetin, spinatoside and jaceidin). There wasa 2.0 fold variation in the total flavonoids (1805-3703 mg/Kg) and 1.7 fold variation in the total antioxidant capacity among genotypes. The correlation between antioxidantcapacity and total flavonoid content was found to be high (0.96).

The medicinal effects may be enhanced in diseases such as cancer by usingantioxidant cocktails (RavitHait-Darshan *et al* 2009).[18] There was a synergisticantioxidant activity between commercial antioxidants and natural antioxidant NAO ((NAO is a unique, powerful antioxidant isolated from spinach leaves). Thecommercial antioxidants used were 3 polyphenols- ferulic acid, caffeic acid and epigallocatechin-3-gallate (RGCG).

Effect of Spinach consumption on serum antioxidant status

Epidemiological studies have clearly established that fruit and vegetableconsumption is associated with better health. Antioxidants present in fruits and vegetables are believed to be responsible for this. However the overall antioxidant status in humans as affected by fruit and vegetable consumption is not very clear.

There was a 7-25% increase in serum antioxidant capacity during 4-h period afterthe consumption of spinach, strawberries, red wine or vitamin C (Cao G *et al* 1998).[19] The *absorption* of phenolic compounds in these foods might have resultedin the increase in antioxidant capacity.

Organic Spinach

Organic farming does not use chemicals during food production, processing orstorage. So the pest pressure may put greater stress on the synthesis of a plant'schemical defense mechanism (Carl and Davis 2006).[20] So, more secondary plantmetabolites are expected to be produced in organic plants. These secondary plantmetabolites, which are mostly antioxidants, may also benefit human health. Somestudies have shown that organic foods contain more antioxidants, although there isno conclusive evidence to prove or disprove this claim.

The Environmental Working Group has reported that spinach is one of the dozenmost heavily pesticide-contaminated produce (EWGs 2011 Shoppers Guide to Pesticides in Produce)[21] Permethrin, dimethoate, and DDT are common pesticidesfound on spinach. It will be interesting to know if these pesticides have any influenceon the nutritional composition or bioavailability of nutrients present in spinach.

Ren *et al* (2001)[22] have compared the antioxidant activity of five organicallycultivated and generally cultivated vegetables (welsh onion, quiang-gen-cal, spinach, chinese cabbage, green pepper). The results reveal that organically grown spinachhas 120% higher antioxidant activity compared to generally cultivated spinach. Atleast two flavonoid contents were more than double (significant at 95% level in t test) in organically grown welsh onion, quiang-gen-cal and spinach as revealed by LC/MS quantitative analysis compared to generally cultivated vegetables.

A comparative study by Eunmi Koh *et al* (2012).[23] has shown that the mean levels ofascorbic acid and flavonoids were significantly ($P<0.001$) higher in organically grownspinach where as nitrate content was significantly ($p<0.001$) higher in conventionalspinach and there was no significant difference in oxalate content in organic andconventional spinach.

Influence of spinach consumption on some patho and physiological conditions: A number of studies have indicated that dailyconsumption of spinach has a positive influence on various organs of human body. These have been categorized under the following headings.

Spinach and age related macular degeneration:

The leading cause of irreversible blindness among adults is age-related maculardegeneration (AMD). Epidemiological studies have revealed an inverse associationbetween AMD and consumption of carotenoid rich foods. Johana et al (1994)[24] haveshown that higher frequency of spinach or collard greens intake substantially lowersthe risk for AMD (*P* for trend <.001). But the intake of preformed vitamin A (retinol) orvitamin E or vitamin C did not reduce the risk for AMD.

Spinach and cataract: Consumption of spinach and other greens at least 5 times/week resulted in 47% lower risk for cataract extraction compared to less than once month consumption(Hankinson *et al*.1992).[25] A retrospective case control study in Northern Italy byTavani *et al* (1996)[26] has also given similar results. They found an inverseassociation between spinach and cruciferae consumption and cataract risk in 207cataract extraction patients and 706 controls. There was 40% less likelihood ofcataract extraction in individuals who consumed spinach at least occasionallycompared to those who never ate spinach.Suzan *et al* (2000)[27] have shown that dietary sources of lutein, particularly spinach and dark green leafy vegetables were most consistently associated with protectionagainst cataract.

The causes for cataract and age-related macular degeneration include bothenvironmental and genetic factors and studies do indicate that dietary factors, particularly antioxidant vitamins and xanthophylls may reduce the risk of thesedegenerative eye diseases.

Spinach and CVD

Spinach is a rich source of fibre. So it has cholesterol lowering effect. HPLC analysisof pepsin−pancreatin digest of spinach Rubisco showed the presence of four newinhibitory peptides for angiotensin I-converting enzyme (ACE) [Yang *et al* (2003)].[28] They are MRWRD, MRW, LRIPVA, and IAYKPAG. The antihypertensive effect ofthese inhibitory peptides on rats is shown below (table 5).

Satoh *et al* (1995)[29] have studied the effect of spinach leaf protein concentrate (SPCC) on serum cholesterol and amino acid concentration in rats fed a cholesterolfreediet. At lower dietary fat level (2%), SPCC had a strong cholesterol loweringeffect. This is due to inhibition of cholesterol and bile acid absorption by the intestineand increase in the concentration of some serum amino acids.

Spinach is one of rich sources of betaine which has a role in protecting internalorgans, improve vascular risk factors and enhance performance (Stuart Craig(2004).[4]Olthof and Verhoef (2005)[30] have shown that rise in homocystein levelsafter meals can be reduced by foods rich in betaine.Increase in homocysteine levelscan result in the development of CVDs. Therefore betaine rich diet may reduce therisk of CVD but it also adversely affects serum lipid concentration, which increasesthe risk for CVDs. So its exact role is not yet clear.

Spinach and Gout

Intake of high protein foods and purine rich foods are considered to be risk factorsfor gout. A 12 year period prospective study (Hyon K Choi *et al* 2004)[31] on 47,150 men for a period of 12 years, has shown that there is no increase in the risk of goutby consuming purine-rich vegetables like spinach in moderate amounts.

Spinach and Kidney Stones

A study has shown that eight foods —spinach, rhubarb, beets, nuts, chocolate, tea, wheat bran, and strawberries—significantly increase the risk of calcium oxalatekidney stones. (Linda K Massey *et al* 1993).[32] Although for healthy peopleendogenous metabolic synthesis is responsible for most of the oxalic acid excretedin the urine, kidney stone formers are advised to reduce the consumption of foodswith high oxalic acid content.

Spinach and Brain Function

The volume of infarction in the cerebral cortex reduced significantly and post-strokelocomotor activity increased significantly in rats which received blueberry, spinach, orspirulina enriched diets (Yun Wang *et al* (2005).[33] This effect was not directlymediated through changes in physiological functions as no difference in bloodbiochemistry, blood CO2, and electrolyte levels were found. Authors found that there was significantly lower caspase-3 activity in the ischemichemisphere in rats receiving blueberry, spinach or spirulina enriched diet. So theysuggest that ischemia / reperfusion-induced apoptosis and cerebral infarction can bereduced by chronic treatment with blueberry, spinach, or spirulina enriched diets.

Spinach and Aging

Studies have shown that CNS functional declines in aging and age related neurodegenerative diseases are due to increased vulnerability to oxidative stress.Antioxidants are believed to play a role in reducing this stress.Onset of age related neural signal-transduction and cognitive behavioral deficits inrats can be retarded by long term dietary strawberry, spinach or vitamin Esupplementation (Joseph *et al* (1998)).[34] In this study following parameters wereexamined. 1) Oxotremorine-enhanced striatal dopamine release 2) Cerebellar betareceptor augmentation of GABA responding 3) Striatal synaptosomalCa 2+Clearance 4) Carbachol-stimulated GTP ase activity 5) Morris Water Mazeperformance. There was a greatest reduction of age related effects on all aboveparameters except parameter 4 in spinach fed rats. Strawberry had the maximumreduction in parameter 4. The authors opine that age related changes on brainfunction can be prevented and sometimes even reversed by nutritional interventionwith fruits and vegetables.

Spinach and Cancer: Among the common vegetables, spinach has the highest in vitro anti-proliferativeactivity on Hep G2 human liver cancer cells. The bioactivity index BI, calculated asBI= ½ (Total antioxidant activity score+ anti proliferative activity score) for dietarycancer prevention was highest for spinach. The authors opine this is not solely dueto the phenolic contents. It might be due to unique phytochemicals present inspinach (Yi-Fang Chu *et al* 2002).[35]

Longnecker *et al* (1997)[36] have shown that the risk of breast cancer can be reducedby regular intake of carrots, spinach and supplements containing Vitamin A. Theyfurther found that eating carrots or spinach more than twice weekly compared to nointake was associated with an odds ratio of 0.56 (95% confidance, Interval 0.34-0.91). The data shows a protective association between intake of carrot and spinachand risk of breast cancer although they do not distinguish among several potentialexplanations for the relation.

Thirteen compounds which acted as antimutagens were purified by Edenharder *et al*(2001)[37] by preparative and micropreparative HPLC from a methanol/water (70:30,v/v) extract of dry spinach. These compounds acted as antimutagens against thedietary carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline in *Salmonellatyphimurium*TA 98.

Eiichi Kotake-Nara et al (2001)[38] investigated whether various carotenoids present infoodstuffs were potentially involved in cancer preventing action on human prostatecancer. The effect of neoxanthin from spinach on reduction in cell viability is shown in Table 6.

Neoxanthin was found to reduce cell viability through apoptosis induction in thehuman prostate cancer cells. The results indicate that the risk of prostate cancer canbe reduced by ingestion of spinach which is rich in neoxanthin.Monogalactosyldiacylglycerols (MGDG) 1 and 5, have been isolated from freshspinach leaves by bioassay-guided fractionation as a galactolipid with possiblecancer preventive effects. MGDG has inhibitory effects on tumor promoter-induced Epstein-Barr virus (EBV) activation (Lars P. Christensen, 2009).[11] Abraham NyskaLiatLomnitski *et al* (2001)[39] showed that skin papilloma counts in female hemizygousTg AC mice could be reduced significantly ($P<0.01$) when treated topically with a Natural Antioxidant from spinach (NAO).

CONCLUSION

Spinach is a rich source of micronutrients, phytochemicals and functional ingredients such as lutein, betaine, flavonoids, neoxanthin, galactolipids. There is enough scientific literature to show that regular consumption of spinach substantially lowers the risk of age related macular degeneration, one of the leading causes of irreversible blindness among adults. There is an inverse association between spinach consumption and cataract risk. Spinach leaf protein concentrate has a strong cholesterol lowering effect in rats. Age related changes on brain function can be delayed by long term consumption of spinach. It has high anti proliferative activity on cancer cells. However, people prone to oxalic acid stones are to reduce consumption of spinach. So quantity and frequency of spinach consumption can be increased.

ACKNOWLEDGEMENTS

This work is part of the project work under Summer Research Fellowship Program2011 of Indian Academy of Sciences, Bangalore, Indian National Science Academy,New Delhi and The National Academy of Sciences, Allahabad. The authors arethankful to Dr A G AppuRao and Dr

Sridevi Annapurna Singh of the Department ofProtein Chemistry and Technology of CFTRI, Mysore for their valuable inputs inpreparation of this manuscript.

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

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List of Tables

Table 1: Xanthophyll composition of spinach

Xanthophyll	Quantity(mg/100g dry weight)
Neoxanthin	58.00+/-4.6
Violaxanthin	65+/- 9.3
Lutein	77.58+/-6.6
Zeaxanthin	1.51+/-0.4
Total xanthophylls	202.09+/-20.9

Source: Rangaswamy L et al 2005

Table 2: Functional ingredients of spinach

Functional ingredient	Quantity
Lutein	586.9µg/Kg@, 76mg/kg#
Betaine	60-64.5 mg/Kg**
Flavonoids	1805-3705 mg/Kg*
Neoxanthin	25.4 mg/kg#
Galactolipids	3300-3880(mg/kg)***

Sources: * Mi Jin Cho et al (2008), **Zeisel S H 2003) , *** Wang R(2002), @Hart and Scott (1995),# Kidmose u et al (2001).

Table 3: Estimated relative bioavailability of β carotene and lutein from spinach in different forms

Spinach form	β carotene (%)*	Lutein(%)*
Whole leaf	5.1	45
Minced leaf	6.4	52
Liquefied spinach leaf	9.5	55
Liquefied Spinach + Dietary fiber	9.3	54

*compared to carotenoid supplement

Source: Jacqueline et al 1999

Table 4: Total antioxidant activity of spinach by various methods

Method used for analysis	Total antioxidant activity	Reference
ORAC	1.94 m mol TE/kg	a
FRAP	9.8 m mol/kg, 26.94 m mol Fe^{++}/l 43-94 m mol TE /kg(dry weight)	b c e
ATBS	8.49 m mol TE/kg	a
TRAP	5.79 m mol TE/kg	c
TOSC	42.20 m mol TE/kg	d
TEAC	8.49 m mol TE/kg	c

TE: Troloxequivalent

a-Pancheon- Fernandez et al 2008, b-Halvorsen L Bente et al 2002, c-Pellegrini N et al 2003

dChuY F (2002), e Boxin O et al 2002.

Table 5: Antihypertensive effect of inhibitory peptides isolated from spinach

Inhibitory peptide	Dosage(mg/kg)	Time for maximum reduction(hours)
MRW	20	2
MRWRD	30	4
IAYKPAG	100	4
LRPVA	100	No reduction

Table 6: Effect of neoxanthin on cancer cell viability

Type of prostate cancer cell	Percent reduction in cell viability
PC 3	10.9
DU 145	15
LNCaP	0

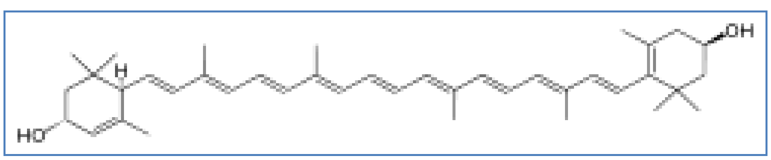

Fig 1: General structure of Lutein

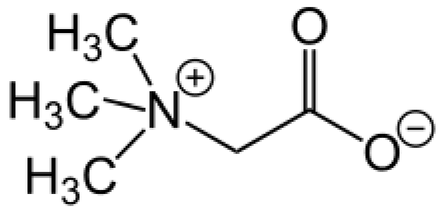

Fig 2: The chemical structure of trimethylglycine

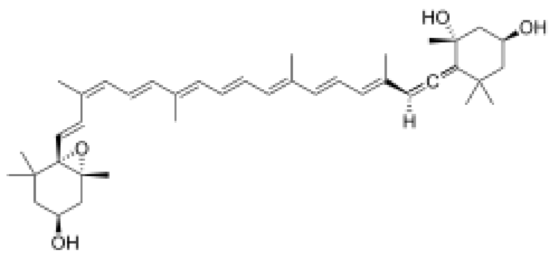

Fig 3 Chemical structure of Neoxanthin

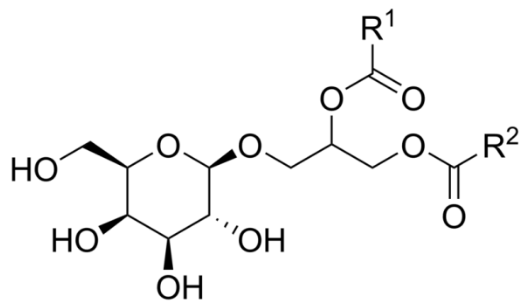

Fig 4: General chemical structure of a monogalactosyldiacylglycerol (MGDG), a type of galactolipid