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Isolation of a biologically active component from the flowers of *Moringa oleifera*

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ABSTRACT

Quercetin-3-O-glycoside, an active flavanol glycoside was isolated from the flowers of *Moringa Oleifera*. The structure of flavanol glycoside was confirmed using FTIR, ^{1}H NMR, ^{13}C NMR and its biological activity namely antibacterial effect was studied by a standardised filter paper disc agar diffusion procedure known as Kirby- Bauer method on *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Entrobacter aerogenes, Proteus mirabilis* and *Bacillus species* using Tetracycline as standard. The results shows that the flavanol glycoside isolated is active against bacteria.

KEY WORDS: Quercetin-3-O-glycoside, Flavanol glycoside, *Moringa Oleifera*, Antibacterial effect.

1. INTRODUCTION:

Moringa Oleifera commonly referred to as the 'drumstick tree' (describing the shape of its pods) or 'horseradish tree' (describing the taste of its roots), is a member of the Moringaceae family which grows throughout most of the tropics and is native to the sub-Himalayan tracts of north-west India, Pakisthan, Bangladesh and Afghanistan. It is a small tree with thick grey bark, fragrant white flowers and long green pods which give the tree the name. *Moringa Oleifera* has been an ingredient of Indian diet since several centuries. It is cultivated almost all over the country and is used as vegetables. Almost all parts of the plant have been utilized in traditional medicine practices. This plant has been reported for its anti-tumor, hypotensive, antioxidant and anti-inflammatory property. The fruits, seeds, leaves and flowers are eaten as nutritious vegetables in some countries. The secondary metabolites present in the plants are reported for curing many disorders. Though they possess diversity in their structure they generally have phenolic groups. Polyphenolic compounds generally includes flavanoids, flavanols, flavanonaes and flavanoid glycosides. The polyphenolic compounds are reported to have biological activities. In view of this the present study has been under taken to isolate the active component of flowers of *Moringa Oleifera* .The structure was confirmed using analytical techniques and the antibacterial activity of the isolated polyphenolic compound was studied.

2. MATERIAL AND METHODS

2.1. Collection of plant material: The flowers of *Moringa Oleifera* was collected from Kumbakonam, Tamil Nadu during the month of December 2014 – March 2015. Using standard floras and standard reference, the specimen was confirmed. The flower petals were separated and used for study (Fig 1&2).

2.2. Reagents: The reagents methanol, light petrol, diethyl ether and ethyl acetate of analar grade were purchased. The reagents were distilled to improve the purity of the reagents. The nutrient agar was purchased and used as such. The microorganisms namely *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Entrobacter aerogenes, Proteus mirabilis* and *Bacillus species* were collected from Government Medical College and Hospital, Thanjavur. Tetracycline (30 mcg) used as standard was obtained from Sigma Aldrich.

2.3. Isolation of flavanol glycosides: The flower petals separated from the flower (1kg) were extracted with methanol (3L) for 72 h. The extract was concentrated to remove excess methanol. It was eluted with light petrol followed with diethyl ether and ethyl acetate. The resulting fraction was confirmed as a single fraction with TLC. The UV spectra of the light yellow coloured powder was recorded on Hitachi – U -2000 – UV –Visible spectrophotometer. The ^{1}H NMR spectra in DMSO – d6 on Brucker DRX – 300 at 400 MHz and ^{13}C NMR spectra at 100 MHz in DMSO –d6 were recorded.

2.4. Antibacterial activity: A standardized filter paper disc – agar diffusion procedure (known as Kirby – Bauer method) frequently used to determine the drug susceptibility of microorganism isolated from an infectious person. This method allows for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that results from the diffusion of the agent into the medium surrounding the disc. In this procedure, sterile disc (200 µl) capacity of uniform sizes were impregnated with 40 mg/ml concentration of different samples and then placed on the surface of Muller – Hinton agar plate that has been seeded with the organism to be tested and the plates were incubated at 37°C for 24 h. After incubation the plates were examined for the zone of inhibition which was indicated in millimeter. Its size was compared with a standardized chart.

3. RESULTS AND DISCUSSION

3.1. Isolation of flavanol glycoside: The flower petals were separated from the flowers. By increasing the polarity of solvent from methanol to ethyl acetate the active ingredient was isolated. The fraction obtained was purified by

TLC. The yellow fraction obtained gave red colour with magnesium and hydrochloric acid. Filter paper soaked with ethyl acetate fraction turned yellow on exposure to ammonia. It didn't responded to Horhammer – Hansel test but it responded to Wilson's boric acid test, Gibb's test and Molisch test.

3.2. UV spectrum: The UV spectrum of the compound in methanol showed two major absorption bands at 233 and 364 nm which are typical for flavanols. The presence of 4' OH group was represented by the degradation of band I with sodium hydroxide. The presence of a free hydroxyl group at position 5 was exhibited by the bathochromic shift of 13 nm in band II with aluminum chloride. The peak between 320 and 335 nm indicated the presence of OH in 7 th position with sodium hydroxide. The shift in band I (13 nm) indicates the presence of diol group in ring B. Molisch test confirms flavanol as flavanol glycoside with a substitution on third OH group. The aqueous solution when neutralised with barium carbonate and Rf values were tabulated with glucose as authentic. (Table.1)

3.3. ^{1}H NMR: The proton at C6 resonated at δ6.189 ppm. The C8 protons appeared as a doublet at δ6.818 ppm (J=2.1 Hz). The C 5′ showed up as a doublet at δ6.846 ppm (J=7.1 Hz). The C2′ and C6′ protons overlapped as a doublet at δ7.559 ppm (J=2.0 Hz). The hydroxyl protons at C5, C7, C3′ and C4′ resonated at δ12.637 ppm, δ10.857 ppm, δ9.314 ppm and δ9.221 ppm respectively. The H 1" of the glucose resonated at δ5.289 ppm, the rest of the sugar protons appeared between δ3.40 ppm and δ3.346 ppm. Fig 3

3.4. ^{13}CNMR: Analysis of ^{13}CNMR data and a complete assignment was given in Table 2. Due to glycosylation at third position, C2 and C4 carbons absorbed at δ156.385 ppm and δ77.518 ppm respectively. C1″absorbed at δ100.920 ppm. The rest of the carbons of the sugar unit appeared between δ77.248 ppm and δ60.647 ppm. Based on the analytical data obtained the glycoside is assigned as Quercetin 3-O-glucoside (Isoquercitrin).Fig 4

3.5. Antibacterial activity: The antibacterial activity of the isolated flavonol glycoside Quercetin 3-O-glucoside was studied by Kirby-Bauer method against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Entrobacter aerogenes, Proteus mirabilis* and *Bacillus species.* The activity of the sample is given in Table 3. Tetracycline was used as standard.

Table.1. Rf (*100) Values of the sugar from the glucoside from flowers of *Moringa Oleifera*
(Whatmann No 1, Ascending, 30± 2°C)

Compound	Developing solvent G	Developing solvent H	Developing solvent I
Sugar from the hydrolysate of the ethyl acetate fraction of *Moringa Oleifera*	16	38	57
Glucose (Authentic)	17	38	58

G: n-BuOH: AcOH:H_2O (4:1:5)(Upper phase); H: Phenol saturated with water; I: t-BuOH:AcOH:H_2O (3:1:1)

Table.2.^{13}C NMR Data and their assignment for the glycoside from the flowers of *Moringa Oleifera*

Compound	Isoquercitrin from literature (δ ppm)	Glycoside (δ ppm)	Compound	Isoquercitrin from literature (δ ppm)	Glycoside (δ ppm)
C-2	156.5	156.385	C-3′	144.8	144.868
C-3	133.7	133.383	C-4′	148.5	148.519
C-4	177.6	177.518	C-5′	116.5	116.281
C-5	161.3	161.306	C-6′	121.6	121.666
C-6	98.8	98.724	C-1″	101.4	100.920
C-7	164.2	164.164	C-2 ″	74.3	74.154
C-8	93.6	93.568	C-3″	76.8	76.555
C-9	156.5	156.385	C-4″	70.3	69.984
C-10	104.2	104.055	C-5″	77.5	77.620
C-1′	121.6	121.666	C-6″	61.3	61.031
C-2′	115.3	115.274			

Table.3.Antibacterial activity of Isoquercitin from the flowers of *Moringa Oleifera*
Capacity of the disc used: 40 mg/ml

Name of the Organism	Tetracycline (30 mcg)	Isoquercitin
Escherichia coli	+	++
Staphylococcus aureus	+++	+++
Klebsiella pneumonia	+++	+++
Pseudomonas aeruginosa	+++	++
Enterobacter aerogenes	+++	+++
Proteus mirabilis	+++	++
Bacillus species	+++	++

+ - less sensitive; ++ - intermediate; +++ - sensitive

Fig.1.*Moringa Oleifera*

Fig.2.Flowers of *Moringa Oleifera*

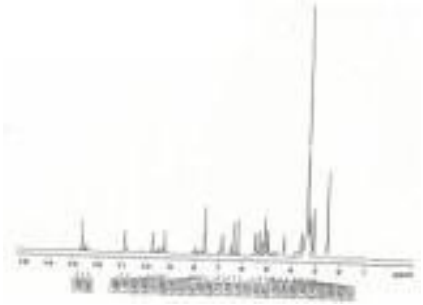

Fig.3.^{1}H NMR of Isoquercitin

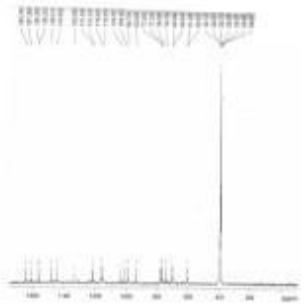

Fig.4.^{13}C NMR of Isoquercitin

4. CONCLUSION

Isoquercitin isolated from the flowers of *Moringa Oleifera* possess intermediate activity thus the sensitivity may be improved by increasing the concentration. Further studies may done to read the bioefficacy of the product.

5. ACKNOWLEDGEMENT

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