

Efficacy of *Mangifera indica* L. seed kernel and *Commiphora myrrha* (Nees) Engl. gum extracts as potent adenosine A₁ receptors

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Abstract— Although specific evaluation of the antimicrobial and anti-inflammatory efficacy of *Mangifera indica* L. and *Commiphora myrrha* (Nees) Engl. have been widely reported, their potential as adenosine A₁ receptors were not investigated. The proposed study envisages evaluating the isolated compounds from the *Mangifera indica* L. (mango) seed kernel and myrrha gum by bioassay guided fractionation process using the adenosine A₁ receptor binding assay in correlation with their anti-inflammatory activity and characterize them by spectroscopy techniques.

The isolated compounds; hydroxy-xanthone-glucoside and quercetin-3-sulphate from *Mangifera indica* (*M. indica*) seed and quercetin from *Commiphora myrrha* (*C. myrrha*) gum exhibited high percentage of displacement of [³H]-DPCPX radioactive ligand, and therefore showed high affinity to A₁-receptor with K_i values in the micromolar range.

Index Terms— *Mangifera indica*, *Commiphora myrrha*, isolated compounds, adenosine A₁ receptor, anti-inflammatory.

I. INTRODUCTION

Adenosine Fig (1) is a potent anti-inflammatory agent, acting at its four G-protein coupled receptors. In humans, there are four adenosine receptors A₁, A₂A, A₂B, and A₃, which detect local changes in adenosine concentration. Each is encoded by a separate gene and has different functions. They have ability to either stimulate or inhibit adenylate cyclase activity [1]. It contributes to anti-inflammatory reactions via effects on neutrophil and endothelial A₁ receptors. This is done by augmenting responses to microbial stimuli, adhesion to endothelium, phagocytosis, and release of reactive oxygen intermediates [2].

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Selective inhibition of the synthesis of A₁ receptors (A₁-R) with anti-sense oligonucleotides confirmed that these receptors are involved in an animal model of asthma. There was remarkable reduction in the number of A₁ receptors in the lung and attenuation of airway constriction to adenosine, histamine, and dust-mite allergen. Although the site of action remains to be determined, selective antagonism of A₁ receptors offers a possible new approach in asthma therapy [3, 4].

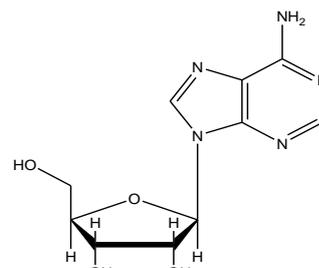


Fig (1) Adenosine

M. indica (Anacardiaceae) and *C. myrrha* (Bursaceae) were selected for adenosine A₁ receptor binding assay according to their A₁ receptor binding assay according to their activity and ther *M. indica* are used medicinally for wounds in mouth, bronchitis and asthma. Moreover, the maceration apeutic uses in traditional medicine, since most parts of throat and tonsillitis and the oil is used in treatment of amenorrhea, athlete's foot, bronchitis, mouth sores and toothache [5].

Garrido *et al.* [6] assessed the analgesic and anti-inflammatory effects of the extract of bark of *M. indica*. Also, they isolated different polyphenols found in aqueous extract of *M. indica* bark for the antinociceptive and anti-inflammatory actions. The alcoholic extract of *M. indica* seed exhibited anti-inflammatory activity in acute, sub acute and chronic cases of inflammation [7].

The isolated crystalline needles from petroleum ether extract of *Commiphora mukul* (myrrha species), which was identified as steroid showed significant anti-inflammatory activity on rat paw edema [8].

The aim of the present work is to investigate the activity of three potential anti-inflammatory compounds isolated from *M. indica* and *C. myrrha* towards adenosine A₁ receptors.

II. PROCEDURE FOR PAPER SUBMISSION

2.1 Plant materials

M. indica (fruits) and *C. myrrha* (gum) were purchased from local market. The samples were authenticated by Mr. Yahiya Suleiman/ Herbarium of the Medicinal and Aromatic Plants Research Institute (MAPRI), Sudan.

2.2 Plant material extraction and liquid fractionation

Powdered air-dried kernel of *M. indica* (500 g) was extracted by methanol and sonicated at room temperature for 1 hr, and the solution was filtered. The procedure was repeated two times and the filtrates were filtered again. The solvent was removed under reduced pressure. The dried extract was re-dissolved in 300 ml water and was partitioned with hexane (3x300 ml), chloroform (3x300 ml) and butanol (3x300 ml), respectively. The same procedure was followed to the *C. myrrha* gum extraction.

2.3 Phytochemical screening

Phytochemical tests were carried out on the methanolic extract of the seed of *M. indica* and *C. myrrha* gum using standard procedures of plant constituent's identification [9, 10].

2.4 Radio binding assay

Methanol as well as fractions (hexane, chloroform and butanol) obtained from the two studies plant spp. were subjected to radio binding assay for adenosine A receptor using the method described by Lohse *et al.* [11], in which the adenosine A₁ receptor binding assay was achieved with a selective A₁ antagonistic [³H]-DPCPX (8-cyclopentyl-[³H]-1, 3-dipropylxanthine) and a selective A₁ agonist CPA (N⁶-cyclopentyladenosine) as radioactive ligands. The used membrane A₁ was prepared with human adenosine receptor produced in Chinese Hamster Ovary (CHO) cell (in concentration 5 µg/50 µl).

2.5 Column and thin layer chromatography

Column chromatography was performed with silica gel 60 (230-400; mesh in a 5 x 58 cm column and Diaion HP-20 in a 2.5 x 40 cm column. Preparative and analytical TLC was performed using aluminum TLC plates of silica gel 60 F.

2.6 Isolation of compounds

The butanol extract (22 g) of *M. indica* and water extract (15 g) of *C. myrrha* were chromatographed on Diaion HP-20 column and eluted subsequently with 500 ml of H₂O, 25% MeOH, 50% MeOH, 75% MeOH, 100% MeOH and CH₃COCH₃ to give two active fractions for each one, respectively. All fractions exhibited activity in the range of 80% - 99% displacement of the [³H] DPCPX radioactive ligand to adenosine A₁ receptor undergo further purification with preparative TLC using a chloroform/ethyl acetate (7:3) solvent system.

2.7 Structure elucidation

The structure of active compounds was elucidated by NMR and LC-MS technique, as well as determination of their melting point.

2.8 Melting point

Melting points (M.P) of the isolated compounds were determined by using Gallen Kamp apparatus.

2.9 NMR measurement

Each pure compound was dissolved in MeOD for ¹H NMR, in which the spectra was recorded on 500 MHz Bruker DMX 500 Spectrometer. Chemical shifts (δ) are given in ppm.

2.10 APCI Mass Spectrometry

A spectrum was recorded on Agilent LC-MS Spectrometer using probe positive-ion and phenomenex RP 18 (4.6 x 150 mm, 5 micron) column. The mass scan range was 100 – 800 m/z. The solvent system was MeOH: H₂O: Formic Acid (90:10:0.1) with flow rate of 1 ml/minute and injection volume of 10 µl/minute. The mass Spectroscopy data was subjected to the library search using metabolomics query bank.

Results and Discussion

Phytochemical tests of *M. indica* seeds and *C. myrrha* gum revealed the presence of coumarines, terpenes, tannins and flavonoids, beside sterols in *C. myrrha*.

Isolated compounds:

5-hydroxy-xanthone glucoside (A): pale yellow crystals; M.P, 213-215°C; ¹H NMR, δ 7.54 (H-1), δ 7.23 (H-3), δ 6.71

(H-4, H-6), δ 7.04 (H-7, H-8, H-9), δ 5.35 (H-1''), δ 3.20-3.70 (sugar proton); positive - ion APCIMS m/z 374 [M+1]⁺ C₁₉H₁₈O₈ [12, 13].

Quercetin-3-sulphate (B): dark yellow crystals; M.P, 250-253°C; ¹H NMR, δ 6.29 (H-6), δ 6.44 (H-8), δ 7.58 (H-2'), δ 6.54 (H-5'), δ 7.36 (H-6'); positive - ion APCIMS m/z 383 [M+1]⁺ C₁₅H₁₀O₁₀S [13, 14, 15].

Quercetin (C): yellow crystals; M.P, 305-307°C; ¹H NMR, δ 6.17 (H-6), δ 6.45 (H-8), δ 7.89 (H-2'), δ 6.80 (H-5'), δ 6.98 (H-6') [15]; positive - ion APCIMS m/z 303 [M+1]⁺ C₁₅H₁₀O₇ [13, 14].

Radio binding assay

In tracing an anti-inflammatory agent from nature or by chemical synthesis, a guiding biological target whether agonist or antagonist are specified. Antagonists substances are needed to inhibit the pro-inflammatory and inflammatory signals transmitted to cell by adenosine. With respect to those arguments, the adenosine A₁ receptor is used as a biological target for indication of the affinity of the extracts and fractions of *M. indica* and *C. myrrha*.

The methanol and butanol extracts of *M. indica* seeds, and the water extract of *C. myrrha* gum exhibited high percentage of displacement of [³H]-DPCPX radioactive ligand to the adenosine A₁ receptor. Therefore, their high affinity to adenosine A₁ receptor was more than [³H]-DPCPX the known radioactive ligand of the receptor (Table I).

The methanol (100%) and acetone (100%) fractions collected from the butanol extract of *M. indica* seed by Diaion HP-20 column chromatography, and the chloroform(75%) fraction isolated from ethyl acetate extract by silica gel column (chloroform : methanol) were promising towards adenosine A₁ receptor according to their high percentage of displacement. Furthermore, the two fractions methanol (100%) and acetone (100%) isolated from water extract of *C. myrrha* by Diaion HP-20 column were also active to adenosine A₁ receptor (Table II).

Bioassay guided fractionation of *M. indica* methanol extract or butanol using the A₁-R assay resulted in the isolation of two flavonoids; A and B as A₁-R active ligands with Ki values in the micromolar range 1.98 ± 0.9 and 2.81 ± 0.15, respectively (Table III). The low Ki value of the compound shows its high

activity. Therefore; both of them showed high affinity to A₁-R since their Ki values were lower than the previously reported A₁-R antagonist theophylline and caffeine (8.5 and 29 μ M). Also, they approximately showed similar Ki values to the reference compounds quercetin and luteolin used in this experiment, which were possessed high activity (2.47 ± 0.67 μ M and 1.66 ± 0.69 μ M, respectively). Moreover, the high activity of A₁-R antagonist A was referred to naphthoflavone, the most active compound with Ki value 0.79 μ M and that was interpreted to structure activity relationship.

The differences of Ki value of these compounds refer to the different radioactive ligands used in the experiments, as well as the concentration of CPA (N⁶-cyclopentyladenosine) and total volume of the tested mixture used in the assay protocol (16, 17).

Similarly, fractionation of *C. myrrha* methanol extract using the A₁-R assay resulted in the isolation of flavonol; C as A₁-R active ligand with Ki values 2.95 μ M in the micromolar range and in agreement with reference compound (Table III).

III. HELPFUL HINTS

Table I Radio binding assay of *M. indica*, *C. myrrha* extracts Activity %

Extract	Activity %				
	MeOH	Hex	CHCl ₃	BuOH	H ₂ O
<i>M. indica</i>	75.69	57.35	36.25	63.36	39.10
<i>C. myrrha</i>	-25.99	44.87	-12.55	-	104.05

Table II Radio binding assay of *M. indica*, *C. myrrha* fractions Activity %

Fraction	Activity %		
	MeOH100%	Acetone 00%	CHCl ₃ 75%
<i>M. indica</i>	98.00	80.00	93.60
<i>C. myrrha</i>	99.00	98.00	-

Table III Ki values of the compounds isolated from *M. indica* and *C. myrrha*

Plant	Compound	Ki (means± S.D) M (μM)
<i>M. indica</i>	A	1.98 ± 0.9

	B	2.81 ± 0.15
<i>C. myrrha</i>	C	2.95 ± 0.24
reference compound	Luteolin	4.89 ± 0.21
	Quercetin	2.97 ± 0.15

K_i = Dissociation constant of ligands for the receptor

IV. EDITORIAL POLICY

I state that the co-author is aware and approve of the submission of our manuscript entitled “**Efficacy of *Mangifera indica* L. seed kernel and *Commiphora myrrha* (Nees) Engl. gum extracts as potent adenosine A1 receptors**” which has been sent to you for possible publication. We hope that it will be up to your standard.

V. CONCLUSION

The methanol extract of *M. indica* seed showed high percentage of displacement of [³H]-DPCPX radioactive ligand. Two compounds from *M. indica* seed undergo adenosine A₁ receptor binding assay and they gave a remarkable activity with K_i values in the micromolar range. The water extract of *C. myrrha* exhibited high percentage of displacement 104% towards adenosine A₁ receptor assay, and its isolated compound quercetin showed high activity with K_i value 2.95 μ M.

The activity of most compounds is relatively relevant in compared to endogenous ligand basal concentration that is needed to activate the receptor.

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