

Identification of human cyclooxygenase-2 inhibitors from *Cyperus scariosus* (R.Br) rhizomes.

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Abstract:

Cyperus scariosus (R.Br) belongs to the family Cyperaceae and it has a diverse medicinal importance. To identify human cyclooxygenase-2 (COX-2) inhibitors from *C. scariosus*, the rhizome powder was exhaustively extracted with various solvents based on the increasing polarity. Based on the presence and absence of secondary metabolites, we have selected the methanolic extract to evaluate the anti-oxidant and anti-inflammatory activity. The same extract was further subjected to gas chromatography-mass spectroscopy (GC-MS) analysis to identify the active compounds. Binding affinities of these compounds towards anti-inflammatory protein COX-2 were analyzed using molecular docking interaction studies. Phytochemical analysis showed that methanol extract is positive for all secondary metabolites. The antioxidant activity of the *C. scariosus* rhizomes methanolic extract (CSRME) is half to that of ascorbic acid at 50 µg/ml. The anti-inflammatory activity of CSRME is higher than that of diclofenac sodium salt at high concentration, which is evident from the dose dependent inhibition of bovine serum albumin denaturation at 40 µg/ml-5 mg/ml. GC-MS analysis showed the presence of nine compounds, among all N-methyl-1-adamantaneacetamide and 1,5-diphenyl-2H-1,2,4-triazine form a hydrogen bond interactions with Ser-530 and Tyr-385 respectively and found similar interactions with crystal structure of diclofenac bound COX-2 protein. Benzene-1, 2-diol, 4-(4-bromo-3 chlorophenyl iminomethyl forms hydrogen bond interactions with Thr-199 and Thr-200 as similar to crystallized COX-2 protein with valdecoxib. Collectively our results suggest that CSRME contains medicinally important anti-inflammatory compounds and this justifies the use of this plant as a folklore medicine for preventing inflammation associated disorders.

Keywords: Anti-inflammatory, anti-oxidant activity, cyclooxygenase-2, *Cyperus scariosus*, gas chromatography-mass spectroscopy analysis, molecular docking.

Background:

Inflammation is a disorder involving localized increase in the number of leucocytes and a variety of complex mediator molecules [1]. Inflammation has shown to associate with numerous environmental and genetic factors [2]. Environmental factors include allergens, infectious agents, toxins and chemicals. Whereas, genetic factors include prostaglandins, cyclooxygenases (COX), interleukins, cytokines, tumor necrosis factor alpha and interferon-gamma [3]. Among those, some COXs have shown to play a major role in triggering the inflammation caused by both genetic and environmental

factors. COX are two distinct isoforms, such as COX-1 and COX-2, they have shown to play a vital role in conversion of arachidonic acid to prostaglandins [4]. Generally the expression levels of COX-2 in normal tissues are below the level of detection, but enhanced expression of COX-2 was detected by proinflammatory cytokines, growth factors and exposure of several carcinogens. Therefore, regulation of COX-2 is very important for therapeutic approaches against inflammatory associated disorder. Many natural products have also been identified as COX-2 inhibitors [5]. For example a synthetic compound azoxymethane has shown to inhibit COX-2 mediated

anti-inflammatory and anticancer agent against colon cancer [6]. In the present investigation we have identified one of the widely distributed medicinal plant *Cyperus scariosus*, to treat inflammation associated disorders via COX-2.

Cyperus scariosus (R.Br) belongs to family Cyperaceae, popularly known as Nagaramotha is an important herb in the Ayurveda [7]. In Ayurveda, Nagaramotha is tikta, katu, kashaya and sheetala, pacified deranged kapha, beneficial in the treatment of fever caused by aggravated pitta, in diarrhea, anorexia thirst burning sensation and fatigue [8]. In Southern India, it's essential oil is employed in the perfume industry and the nut grass is used in the formulation of hair and skin care products; it stimulates sebaceous glands near hair roots [9]. The dried tuberous roots of *C. scariosus* are used in traditional medicine [10]. Tubers are credited with astringent, diaphoretic, diuretic, desiccant, cordial, and stomachache properties [11]. In traditional medicine, the rhizomes of the plant are used in the treatment of inflammation [12]. However there is no scientific proof for justifying the traditional use of rhizomes in the treatment of inflammation. Hence, the present work was undertaken to evaluate the anti-inflammatory activity of *C. scariosus* rhizomes.

Methodology:

Collection of plant material

Cyperus scariosus plant material was collected from Sri Satyadeva nursery, Kadiyam, East Godavari district, Andhra Pradesh, India. The plant was taxonomically identified by Dr. A.Prasada Rao, Senior Botanist in K L University, Vijayawada, Andhra Pradesh, India. A voucher specimen has been deposited at K L University Botanical garden (voucher specimen number KLU-1250) for further use.

Preparation of plant extract

Cyperus scariosus rhizomes were separated from the plant, washed with running tap water to remove the dust, followed by sterilization with double distilled water, shade dried and made into a fine powder with a blender. 500 g of the rhizome powder were exhaustively extracted with various organic solvents such as petroleum ether, hexane, chloroform, ethyl acetate, methanol and ethanol with soxhlet apparatus for 12–24 h. The extracts were filtered with Whatman filter paper (type 4) and the filtrate was concentrated under reduced pressure on rota vapor under vacuum (BUCHI, R-3000, Switzerland) at 400°C temperature. The filtrate was used for analysis of phytochemical compounds, anti-oxidant activity and for gas chromatography-mass spectroscopy (GC-MS) studies.

Phytochemical analysis

The various solvent extracts were subjected to phytochemical analysis to investigate the presence or absence of various phytoconstituents such as glycosides, terpenoids, saponins, phytosterols, alkaloids, phenolic compounds, tannins, flavonoids and diterpenes as per the standard methods [13].

Gas chromatography and mass spectroscopy separation conditions

The phytochemicals were analyzed by GC-MS Agilent 5975-C Series instrument employing the electron impact mode (ionizing potential – 70 eV) and a capillary column (DB-5 ms Agilent) (length 30 m × diameter 0.25 mm, film thickness 0.25 µm)

packed with 5% phenyl dimethyl silicone) and the ion source temperature was monitored at 200°C. Further, the GC-MS settings were indicated as the initial column temperature was set at 70°C and kept hold for 2 min; the temperature was increased to 300°C at a rate of 10°C/min for 9 min, and placed in isothermal condition for 2 min. The column oven temperature was maintained at 70°C. Helium was used as carrier gas with 99.9995% purity. Samples were injected at a temperature of about 250°C with a split ratio of 10:1 with a flow rate of helium 1.51 ml/min. Mass scan (m/z): 45–1000, total MS running time: 36 min [14]. The constituents were identified after comparison with those available in the computer library (National Institute of Standards and Technology [NIST] vs. year 2005) attached to the instrument and reported.

Evaluation of total antioxidant potential by phosphomolybdate method

The total antioxidant capacity of the methanolic rhizome extract was evaluated by phosphomolybdate method [15]. A volume of 3 ml of phosphomolybdate reagent is mixed with the series of 300 µl of *C. scariosus* rhizomes methanolic extract (CSRME) of the plant or standard solution or methanol in a test tube. The test tubes were capped with silver foil and incubated in water bath at 95°C for 90 min. Later, the tubes were cooled down to room temperature, and the absorbance measured at 695 nm against blank. Ascorbic acid was used as a standard. The antioxidant activity of CSRME was expressed as µg/ml of ascorbic acid equivalents.

In vitro anti-inflammatory activity using bovine serum albumin denaturation assay

To evaluate the anti-inflammatory activity of phytochemical compounds present in *C. scariosus*, we used an anti-denaturation of bovine serum albumin (BSA) assay [16]. In brief the reaction mixture consist of 0.2 ml (10 mg/ml) of BSA, 2.8 ml of phosphate buffered saline (PBS, pH - 6.4), and 2 ml of varying concentrations of methanolic extracts of *C. scariosus* 50, 100, 200, 400, 800, 1200, 1600, 2000, 5000 µg/ml to a final volume of 5 ml. PBS lacking BSA served as control. The samples were incubated at 37°C ± 2°C for 15 min and then transferred to 70°C water bath for 5 min. After cooling the sample, the turbidity was measured at 620 nm using a spectrophotometer. The anti-inflammatory activity of phytochemical compounds was determined by plotting the percentage of inhibition with respect to control against treatment condition. In the present study diclofenac sodium tablet was used as a positive anti-inflammatory drug. The percentage inhibition of protein denaturation was calculated by using the following formula.

Where, V_t = absorbance of the test sample,
 V_c = absorbance of control.

Protein (receptor) preparation

The three-dimensional protein structures were obtained from protein data bank (PDB). Crystal structure of human COX-2 proteins (PDB ID: 1PXX and 2AW1) and their respective ligands such as diclofenac and valdecoxib were retrieved from RCSB (Research Collaboratory for Structural Bioinformatics) protein databank. Hydrogen atoms were added using free online program reduce ([http://kinemage.biochem.duke.edu / software/reduce.php](http://kinemage.biochem.duke.edu/software/reduce.php)). Then the PDB files are uploaded to make receptor 3.0. Make receptor is a graphical utility program for

creating receptor protein compatible to fast rigid exhaustive docking (FRED 3.0), which uses the structure of a target protein (receptor file) and the structure of a bound ligand to dock and score molecules. Make receptor workflow have four parameters, which include (1) uploading a target PDB, which separates protein from bound ligand (2) creating a box enclosing the

active site using molecular cavity detection followed by (3) shape potential determination and selecting reasonable inner and outer contours (4) detecting and adding amino acid constraints to dock specific interactions with small molecules. Finally, the prepared molecule is saved as a receptor file in OEB format.

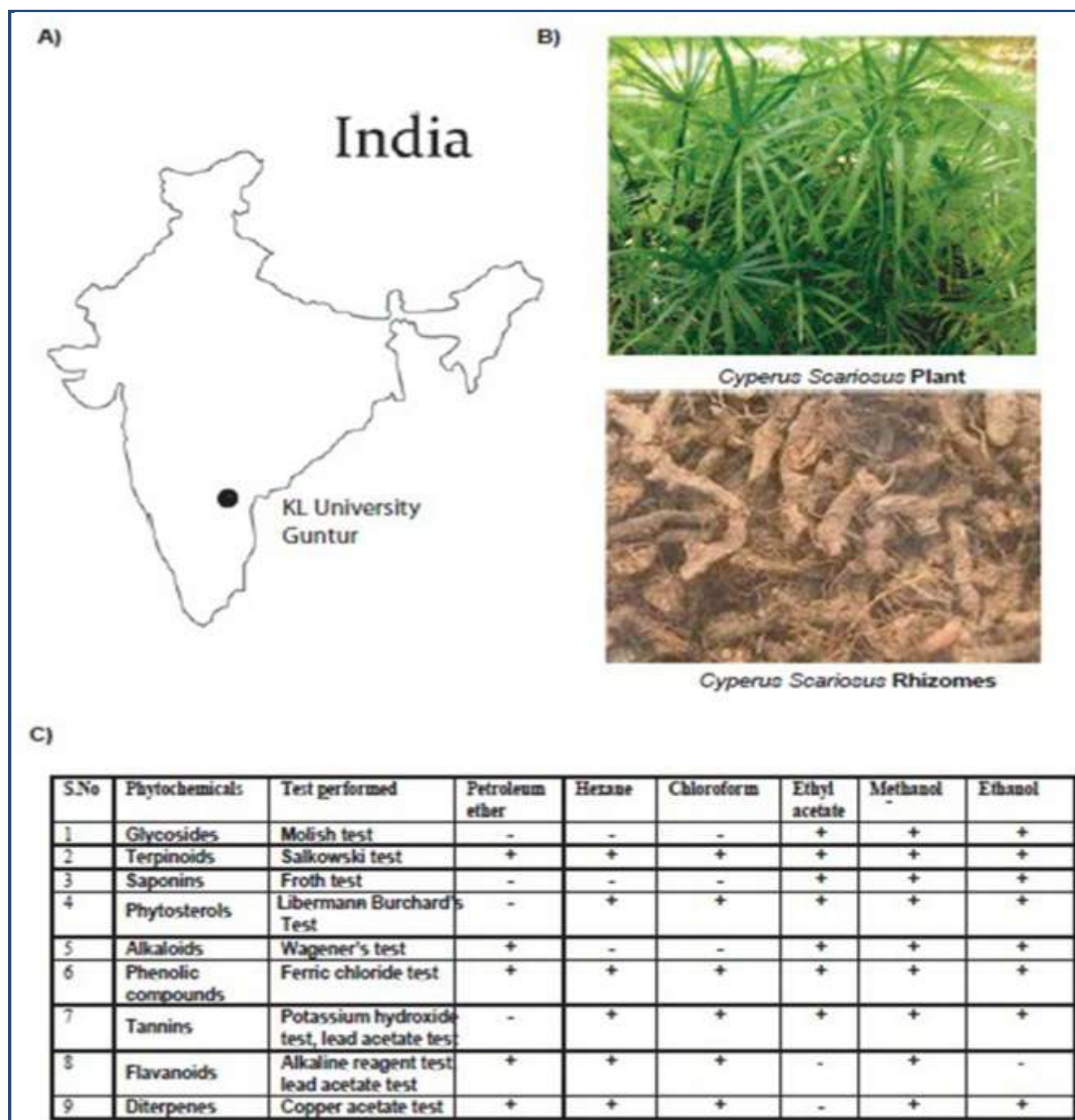


Figure 1: Phytochemical screening of *Cyperus scariosus* plant. a) Geographical representation of *C. scariosus* plant region in India; b) *C. scariosus* (Cyperaceae) plant, rhizomes; c) Phytochemical screening of *C. scariosus* rhizome extracts using various solvents. + and - indicates presence and absence of phytochemicals.

Ligand preparation

Cyclooxygenase-2 inhibitory anti-inflammatory compounds were extracted from (1PXX and 2AW1). The conformational space of the compounds was employed using optimized ensemble generation application (OMEGA) program from Open

Eye Scientific Software, Inc., Santa, NM, USA, (www.eyesopen.com). In our computations we generated a maximum of 500 conformers per molecule as a default using OMEGA 2.4.6 and build as a single database per molecule including *C. scariosus* phytochemical compounds. All possible confirmations of ligand

were generated at physiological pH \pm 4-7. High-throughput docking using fast rigid exhaustive docking Fast rigid exhaustive docking 3.0.0 was used in this study to dock the pre generated multi-conformer library. FRED filters the poses based on adequate contact with the receptor. Fred dock/score all

possible positions of each ligand in the binding site and clash poses with the protein get rejected from the docking analysis. The final poses are scored using chemgauss 4 score as default parameter. The filtered compounds were docked into the binding site of human COX 2 (PDB code: 1PXX and 2AW1).

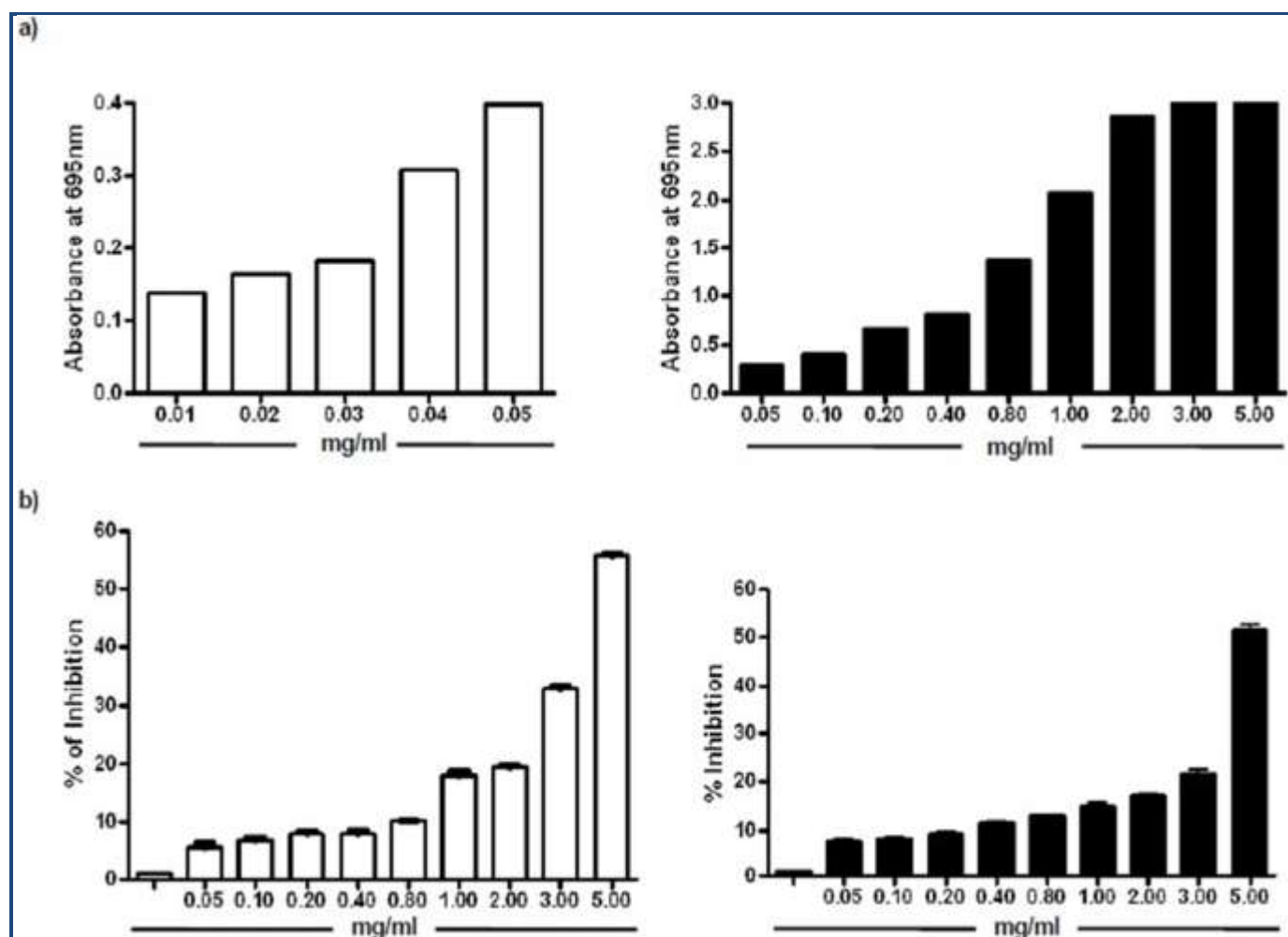


Figure 2: Evaluating medicinal importance of *Cyperus scariosus* rhizomes methanolic extract: **a)** Antioxidant activity of ascorbic acid (left top panel) and plant extract (right top panel); **b)** Anti-inflammatory activity of diclofenac (left bottom panel) and plant extract (right bottom panel).

Absorption, distribution, metabolism, excretion prediction

Qikprop module was used to predict adsorption, distribution, metabolism, elimination and other molecular properties. The foremost criteria for screening ligands are Lipinski's rule of five. In this process various pharmacological, pharmacokinetic, and physiochemical properties were screened [17].

Results:

Identification of phyto-chemical constituents

Phytochemical analysis showed the presence (+) and absence (-) of phytochemical compounds in different solvent extractions. Petroleum ether, hexane, chloroform, ethyl acetate, methanol and ethanolic extraction fractions revealed the presence of phenolic compounds and terpenoids. Phytosterols and tannins are found in all solvents except petroleum ether. Glycosides and saponins shown to be positive in solvents like ethyl acetate, methanol and acetone respectively (Figure 1). Among all solvent extracts, methanol extract hold all

phytoconstituents and therefore it is used for further biochemical analysis.

Gas chromatography-mass spectroscopy analysis of *Cyperus scariosus* rhizomes methanolic extract

The powdered rhizoids of the *C. scariosus* are carefully packed into the soxhlet apparatus and extraction is carried in the presence of 100% methanol. Excess of the methanol were removed by simple evaporation technique. The final fine form of the crystalline powder was sent for GC-MS analysis. The spectrum profile of the GC-MS data of the *Cyperus* was compared with the spectrum of the known components stored in the NIST library. Results showed three major peaks along with remaining nine phytochemical constituents. The peak number one shows retention time at 15.925 min at an area of 376598 with 40.19% area and it gives three best hits from each library such as 1,5-diphenyl-2H-1,2,4-triazoline, 2-Propene-1-one,3-(4-nitrophenyl), phenylacetamide N-ethyl-N-(3-methyl). The second peak showed retention time at 17.087 min at an area

of 402087 with, 42.91% area. This will also give three best hits from each library and the compounds were 6-(2-Aminophenyl)-1, 2, 4-triazine, benzene-1, 2-diol, 4-(4-bromo-3-chloro), thiazolidine-4-one, 2-(4-bromophenyl) respectively. Peak three shows retention time at 19.411 min with an area of 158425 which occupies 16.91% of the area and the third peak also represents

the same number of hits from each library (E)-2-bromobutyloxychalcone, N-methyl-1-adamantaneacetamide, 2-ethylacridine. The name, molecular weight and structure of the components of the compounds were ascertained in **Table 1** (see **supplementary material**).

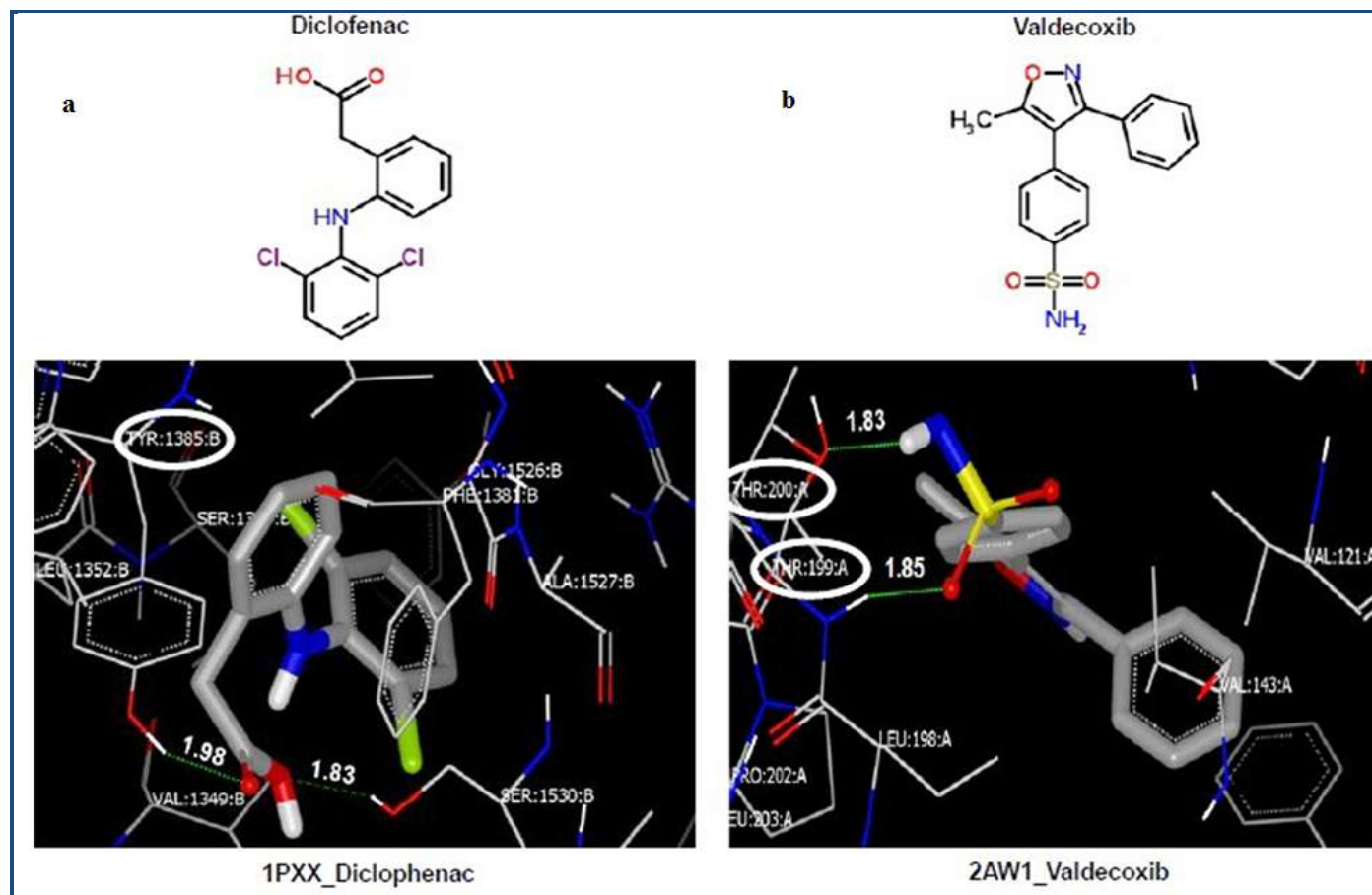


Figure 3: Protein-drug interaction against human cyclooxygenase-2 (COX-2): **a)** Diclofenac; **b)** valdecoxib interactions with human COX-2 (protein data bank ID: 1 PXX, 2AW1). Green dotted lines indicate direct hydrogen bond interaction with flexible aminoacid residues. Numbers indicates the hydrogen bond distance in Angstroms with their respective aminoacid constrains.

Antioxidant activity of Cyperus scariosus rhizomes methanolic extract

The total antioxidant capacity of the CSRME was calculated based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH, which was measured spectrophotometrically at 695 nm. Our results showed that the antioxidant activity of CSRME increases in a dose dependent manner at a concentration of 50 µg/ml–5 mg/ml. However, the concentration above 2 mg/ml does not shown any marginal difference as compared to 5 mg/ml of CSRME. The antioxidant activity of CSRME at 100 µg/ml is similar to ascorbic acid at 50 µg/ml concentration. It suggests that the CSRME contain strong antioxidant activity and attributed due to the presence of phenolic compounds (**Figure 2a**).

Anti-inflammatory activity of Cyperus scariosus rhizomes methanolic extract

Anti-inflammatory effect of CSRME was evaluated by measuring percent inhibition of bovine serum albumin denaturation (BSA). Our results confirm that CSRME inhibits

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the denaturation of BSA in a dose-dependent manner throughout the concentration range of 50–5000 µg/ml. The per cent inhibition of BSA denaturation is enhanced with an increase in the concentration of the plant extract. Diclofenac sodium tablet (50–5000 µg/ml) was used as reference drug which also demonstrate concentration dependent inhibition of protein denaturation. However, at higher concentration, the effect of diclofenac sodium was found to be less as compared with CSRME (**Figure 2b**).

Identification and characterization of the active site amino-acid constraints for human cyclooxygenase-2

To identify the active site amino acid constraints, the three dimensional structural information of the target proteins (1PXX, 2AW1) were retrieved from the RCSB data bank (<http://www.rcsb.org>). As a first step, hydrogen atoms were added to both proteins using reduce-a command line execution program (<http://kinemage.biochem.duke.edu/software/reduce.php>). To identify the location, shape and docking constrains around the active site of bound ligands, grid box was generated using molecular cavity detection algorithm in receptor setup

workflow module at Open Eye software. The box dimensions for crystallographic diclofenac and valdecoxib displayed dimensions as $13.49 \text{ \AA} \times 16.21 \text{ \AA} \times 15.48 \text{ \AA}$ with a box volume of 3383 \AA^3 . Two flexible amino-acid constrains such as Tyr-385

and Ser-530 with diclofenac and Thr-199 and Thr-200 with valdecoxib formed a direct hydrogen bond interaction (**Figure 3**).

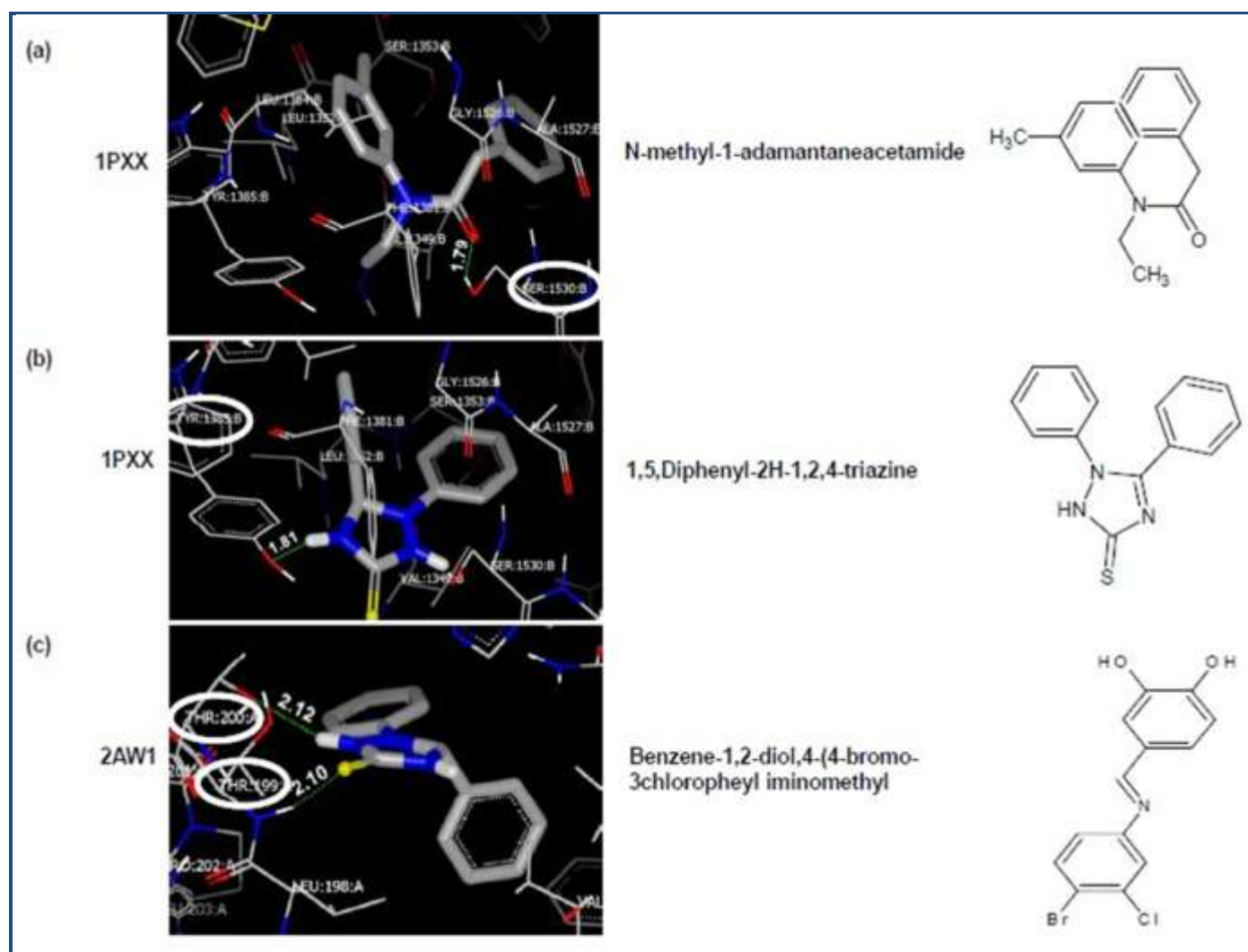


Figure 4: Protein-plant compound interaction against human anti-inflammatory protein cyclooxygenase-2 using molecular docking analysis; **a)** N-methyl-1-adamantaneacetamide with 1PXX; **b)** 1, 5, diphenyl-2H-1,2,4-triazine with 1PXX; **c)** Benzene-1,2-diol,4-(4-bromo-3-chlorophenyl iminomethyl) with 2AW1.

Receptor based molecular docking using a library of small molecule compounds from *Cyperus scariosus* against human cyclooxygenase-2. Based on the importance of diclofenac with human COX-2 and the interactive amino acid constrains, further we decided to search for small molecule inhibitors from *C. scariosus* similar structure activity relationship. For those natural compounds from *C. scariosus* were filtered by applying the expanded Lipinski's drug-likeness criteria: Molecular weight between 150 and 440 Da; the presence of 0–6 hydrogen-bond acceptors and 0–4 hydrogen bond donors; <10 rotatable bonds; and overall hydrophobicity below $\log P - 5.0$. The physicochemical properties of all natural compounds are listed in **Table 1**. Among all N-methyl-1-adamantaneacetamide and 1,5-Diphenyl-2H-1,2,4-triazoline bound deep within a narrow pocket formed by the inner lobe cleft as reported to X-ray crystallographic structures of 1PXX and 2AW1. N-methyl-1-adamantaneacetamide formed one direct hydrogen bond

interaction between Ser-530 with a distance of 1.79 \AA , whereas 1,5-diphenyl-2H-1,2,4-triazoline formed direct hydrogen bond interaction with Tyr 385. On the other hand, benzene-1,2-diol,4-(4-bromo-3-chloro) formed two hydrogen bond interactions with Thr-199 and Thr-200 with a distance of 2.10 and 2.12 \AA , distance as similar to crystal ligand valdecoxib for 2AW1. Some important hydrophobic amino acid residues surrounding the N-methyl-1-adamantaneacetamide, 1,5-diphenyl-2H-1,2,4-triazoline and benzene-1,2-diol,4-(4-bromo-3-chloro) are Ser-353, Leu 384, Leu 352, Tyr 385, Phe 381, Leu 531, Ser-353, Leu 198, Val 121, Val 143, and Ala 527 (**Figure 4**).

Discussion:

The use of traditional medicine by the people for treatment of different ailments is expanding to newer horizons as plants still remain the novel source of structurally important compounds that lead to the development of innovative therapeutics [18].

Enormous number of plants, even though identified, their medicinal values are still with local people and tribal populations [19]. Many herbal preparations are being prescribed as anti-inflammatory and analgesic in the traditional literature [20]. The search for new anti-inflammatory and analgesic agents from the huge array of medicinal plant resources is intensifying [21]. Several drugs has been discovered to inhibit or delay the inflammation of rheumatoid or osteoarthritis by targeting anti-inflammatory protein COX-2. Food and Drug Administration (FDA) approved selective COX-2 inhibitors namely nimesulide, etodolac, and meloxicam showed potent anti-inflammatory compounds in preclinical models. All three compounds selectively bind Ser-530 amino acid residue, which is depicted by crystallographic and molecular docking studies [22]. Very recently second generation drugs have also been developed to target COX-2, considering minimal effects on gastrointestinal or renal problems. However, they could not able to overcome the combined side effects associated with digestive and urinary tract infections [23]. Therefore, plant metabolites are increasingly paying attention over synthetic derivatives against inflammation to reduce side effects. In our continuing efforts, we have identified selective COX-2 inhibitors from *C. scariosus*. Several phytochemical constituents are abundant in the rhizoid methanolic extract, which include alkaloids, tannins, terpenoids, flavonoids and phenol derivatives. It should be noted that phenol components are of importance and interest in pharmacy due to their relationship with antioxidant activity [24]. On the other hand, the rhizomes of *C. scariosus* demonstrate effective inhibition of BSA denaturation. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases [25]. The increments in absorbance of test samples with respect to control indicated stabilization of protein that is inhibition of heat-induced protein (BSA) denaturation by plant extract. It suggests that this plant is the reservoir of potentially useful chemical compounds, which serve as drugs, provide newer leads and clues for modern drug design. Molecular docking approaches are generally used in modern drug design process to understand the protein ligand interactions [26]. The three-dimensional structure of the protein-ligand composite could be served as a considerable source of understanding the proteins that interact with one another and perform biological functions [27]. Hence, knowledge of protein and ligand interactions with the specific drugs may provide a significant insight into the binding interactions and relativeness of the drug [28]. To identify the bioactive compounds present in the *C. scariosus* we have retrieved COX-2 protein co-ordinates from 1PXX and 2AW1. Respective ligands such as diclofenac and valdecoxib were separated from COX-2 and self-docking results were performed to identify the active site amino acid residues. Results showed that diclofenac forms a hydrogen bond interaction with Ser-530 and Tyr-385, whereas, valdecoxib forms a hydrogen bond interaction with Thr-199 and Thr-200. It supports previously published anti-inflammatory activity of diclofenac and valdecoxib toward human COX-2 [29, 30]. The hybrid chemgauss4 score for the diclofenac and valdecoxib is -10.6 and -7.73 Kcal/mol respectively. The docking scores against selected natural compounds showed higher binding affinity towards COX-2, which is evident from the hybrid chemgauss4 score for N-methyl-1-adamantaneacetamide (-11.786 Kcal/mol), 1,5-diphenyl-2H-1,2,4-triazoline (-10.909 Kcal/mol) and benzene-1,2-diol,4-(4-bromo-3-chloro) (-7.806

Kcal/mol) are significantly equal to the diclofenac and valdecoxib. All selected compounds including Diclofenac show molecular weight below 450, hydrogen bond donor (<5) and acceptor (<10), log P ≤ 5, and rotatable bonds ≤ 5 of Lipinski's rule of five [31] Polar surface area (PSA) has shown to inversely correlate with lipid penetration ability [32] Compounds that are completely absorbed by humans tend to have PSA ≤60Å², while compounds with PSA >140 Å² are <10% absorbed. The selected compounds show PSA values in the range of 25–65, suggests that these compounds likely to support the drug-likeness proposed by Lipinski [33] Qualitative human oral absorption showed the score between three and two and is higher than FDA approved anti-inflammatory drugs diclofenac and valdecoxib. All the compounds satisfy the values of partition coefficient of octanol/gas (QPlogPoct) (-2.0–6.5), brain/blood (QPlogBB) (-3.0–1.2) predicted to be blood brain and gut blood barrier. Collectively our results suggest that natural compounds of *C. scariosus* have anti-inflammatory activity and they can be further considered for in vivo experimental study in context to normal and disease state.

Conclusion:

Cyperus scariosus belonging to the family Cyperaceae and methanolic extract of this plant rhizomes (CSRME) is rich in anti-oxidants and anti-inflammatory compounds. *In-silico* molecular docking analysis showed N-methyl-1-adamantaneacetamide, 1,5-diphenyl-2H-1,2,4-triazine and benzene-1,2-diol,4-(4-bromo-3 chlorophenyl iminomethyl) interacted with anti-inflammatory COX-2 binding receptors like 1PXX and 2AW1. It justifies the use of this plant as a folklore medicine for preventing inflammation associated disorders.

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Conflict of Interest: None.

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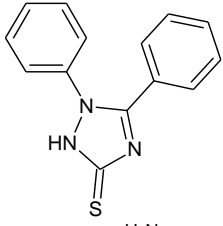
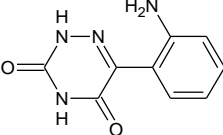
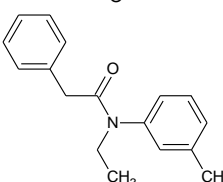
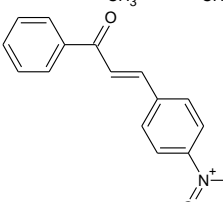
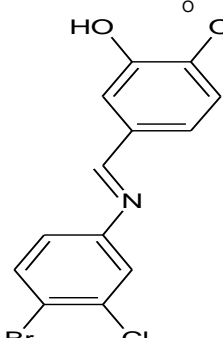
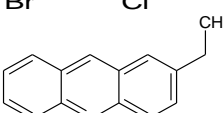
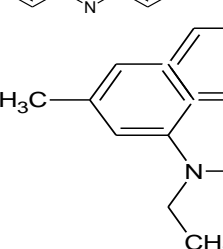
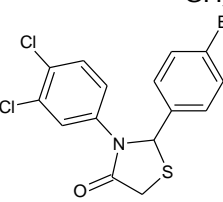
Edited by P Kanguane

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Supplementary material:

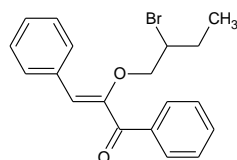
Table 1: Different compounds of *Cyperus scariosus* obtained by GC-MS analysis

Molecule ventilation	Molecular formulae	Molecular weight (Da)	Structure	Smiles
1,5-diphenyl-2H, 1,2,4-triazine	C ₁₄ H ₁₁ N ₃ S	253.3		S=C2/N=C(\N(c1ccccc1)N2)c3ccccc3
6-(2-aminophenyl)-1,2,4-triazine	C ₉ H ₈ N ₄ O ₂	204.1		O=C1C(=N/NC(=O)N1)\c2ccccc2N
Phenylacetamide ethyl-N-(3-methylphenyl)-	N-C ₁₇ H ₁₉ NO	253.3		CCN(C(=O)Cc1ccccc1)c2cc(C)ccc2
2-propene-1-one, 3-(4-nitrophenyl)-1-phenyl	C ₁₅ H ₁₁ NO ₃	253.2		O=[N+](O-)]c2ccc(\C=C\C(=O)c1ccccc1)cc2
Benzene-1,2-diol,4-(4-bromo-3-chlorophenyl iminomethyl)	C ₁₃ H ₉ BrClN ₃ O ₂	326.5		c1cc(c(cc1C=Nc2ccc(c(c2)Cl)Br)O)O
2-ethyl acridine	C ₁₅ H ₁₃ N	207.2		n1c3c(cc2c1cccc2)cc(cc3)CC
N-methyl-1-adamantaneacetamide	C ₁₃ H ₂₁ NO	207.3		CCN(c1cccc(c1)C)C(=O)Cc2ccccc2
Thiazoline-4-one, 2-(4-bromophenyl)-3-(3,4-dichlorophenyl)	C ₁₈ H ₁₁ Cl ₂ N ₃ O ₂	403.1		c1cc(ccc1C2N(C(=O)CS2)c3ccc(c(c3)Cl)Cl)Br

(E)-2-bromo
butyloxychalcone

$C_{19}H_{19}BrO_2$

359.2570



CCC(COC(=Cc1ccccc1)C(=O)c2ccccc2)Br

GC-MS: Gas Chromatography-Mass Spectroscopy
