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ORIGINAL ARTICLE **OPEN ACCESS**

Biopotentiality of Cow Urine Extract of Trikatu Churna: Finding of Effectual Remedy from Ancient Therapeutic Aspects

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ABSTRACT
Natural remedies are always regarded as a substantial therapeutic source for global health. Trikatu churna and cow urine are innate components of the long-established healthcare system of Ayurveda. Long since multitudinous plant materials and cow urine are practised together to accomplish diverse therapeutic roles, there is a need for scientific recognition for such conventional claims. The existing work communicates the in vitro curbing potentiality of cow urine extract of Trikatu churna against oxidants and enzymes. The remarkable antioxidative prospects were observed during DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical bridling scheme sequentially noted with IC50 numerals of 109.96 µg/mL and 115.38 µg/mL. The inquisition of antidiabetic aptitude sequentially introduced the IC50 statistics of 179.11 µg/mL and 143.26 µg/mL during α-amylase and α-glucosidase inhibitory execution while the assessment of anti-inflammatory potentiality by anti-lipoxygenase mode was noticed with the IC50 numeral of 171.77 µg/mL. Delineation of cow urine extract was observed in a poor manner during anti-proteinase perusal. The comprehensive outcomes of the performed study prompted that, conventional claims of herbal treatment in coalition with cow urine may become a purposeful therapeutic way for global healthcare, and competency of cow urine extract of Trikatu is credibly interconnected with the crucial phytochemical substances detected in the extract.
Keywords: *Trikatu, cow urine, antioxidative, antidiabetic, anti-inflammatory*

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INTRODUCTION

Omnipresent oxidative stress was affirmed as a major fundamental cause for the induction of diabetes and intertwined inflammatory plight. Numerous research data pointed out that the substantial stress of oxidants and inflammatory conditions may affect the operative mechanism of islets of Langerhans and boost the genesis of hyperglycemic situation. Protracted hyperglycemia is reported to produce widespread oxidative stress by affecting basal mitochondrial functioning and may elevate the production of inflammatory mediators [1, 2]. Cotreatment of such intertwined health issues and related complications always remains a challenge for health specialists because chemical-based drugs suggested in the therapy of diabetes are linked up with multiple undesired ramifications like hypoglycemic conditions, disturbances in the digestive tract, hypersensitivity, toxicity in the circulatory system, etc. These drugs are not adequate to constrain the dissimilar complications noticed in diabetic sufferers and therefore the course of life of such patients directed with multi-drug remedies [3, 4].

Copious research findings reported that antioxidative fragments from plant sources can competently control oxidative load by neutralizing the oxidative species and forestalling cell damage in the body. These antioxidative fragments are also published as operative against diabetes and associated complications [5]. In India, Ayurveda is the conventionally endorsed medicinal establishment, explaining the application of natural remedies only, and is currently noticed by the twenty-first-century pharma segment [6]. The polyherbal formulation is a noteworthy theory in Ayurveda as due to its multi-therapeutic features. In such formulations, disparate bioactive components can exert additional efficiency through their diverse mode and provide comprehensive beneficial results in patient health [7]. Trikatu churna is a significant ayurvedic admixture, prepared by blending the tantamount powdered quantity of Fruits of *Piper nigrum*, *Piper longum,* and Rhizomes of *Zingiber officinale*. Ayurvedic literature describes the Trikatu churna in the treatment of digestive upset, diseases of the respiratory tract, abdominal tumors, skin diseases, diabetes, and obesity [8].The research world validated the efficacy of Trikatu churna for antioxidative, immune

boosting, antiobesity, antimicrobic, anthelmintic, antihe patotoxic, anti-inflammatory, antitumor, and anti-anorectic effects. The affluent nature of Trikatu churna for the presence of multiple bioactive components fosters its propensity for medicinal value [9]. Cow urine (Gomutra) is one more therapeutically valued remedy instructed in Ayurveda-based literature to treat different ailments associated with the alimentary system, urinary tract system, circulatory system, and derma ailments. It is also documented that Cow urine can drop out complete body imbalances and nourish health. Scientific assessment disclosed the existence of diversified biocomponents like amino acids, essential minerals and salts, hormonal substances, vitamins, and digestive enzymes [10].It is also acknowledged that co-administration of cow urine with herbal products improves the bioavailability of bioactive constituents of these products and hence strengthens the therapeutic potential. [11]. Conventional Ayurveda experts recommended the application of cow urine in combination with different herbal materials to cure multifarious ailments including pyrexia, seizure, bloodlessness, stomachache, constipation, and far more. One of the eminent traditional organizations, 'Gayatri Parivar', prepares antidiabetic herbal products by employing cow urine [12]. In order to investigate the rationality in the back of such conventional therapy, the existent oxidant and enzyme inhibitory experimentation was undertaken by employing the cow urine extract of Trikatu churna.

MATERIAL AND METHODS

Collectionof plant materials and cow urine: *Piper nigrum* and *Piper longum* fruits, and *Zingiber officinale* rhizomes were obtained from the local herbal retailer. The botanical diagnosis of all material was asserted from the Botanical section of Manhorbhai Patel College, Sakoli, District. Bhandara, MS, India. Cow urine was obtained in the early morning time from the local cow breed, filtered with Whatman filter paper, and stored in a volumetric flask for the next experimentation (Fig. 1).

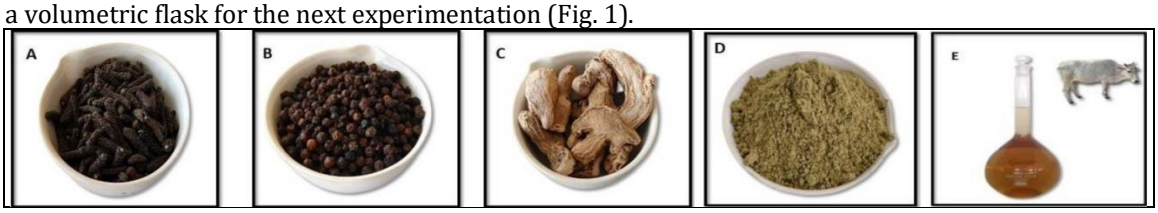

Fig. 1. A: Fruit of *Piper nigrum,* B: Fruit of *Piper longum,* C: Rhizome of *Zingiber officinale,* D: Trikatu churna, E: Cow urine and source

Formulation and Extraction of Trikatu churna: The obtained plant materials were purified, dried out, and triturated individually. All triturated materials were sifted (sieve number 80) and assorted in akin quantities. A 50 g of precisely weighed quantity of formulated Trikatu churna was independently macerated with cow urine (Gomutra) and distilled water for 72 hours, filtered, and evaporated in a thermostatic water bath [13].

Phytochemical scrutiny: In order to ensure the phytochemicals nature of cow urine extract and the aqueous extract of Trikatu churna, miscellaneous qualifying tests were carried out in accordance with the standard protocol (Fig. 2 and Fig. 3) [14].

***In vitro* antioxidative scrutiny**

Anti-DPPH vetting: Vanishing the purple aspect of the DPPH solution is the assessment criteria for the evaluation of antioxidative aptitude. Practically, the discrepant concentrations of each trial sample were separately homogenized into 1000 µL methanolic DPPH reagent (0.2 mM) and laid unlighted for 30 minutes. The collation study was done against the matchable concentrations of ascorbic acid by computing the outcomes at 516 nm [15].

Anti-ABTS vetting: An aptitude of the sample to neutralize the cations of ABTS compound is the appraisement base of the experimented model. In the existent experiment, ABTS radicals were evolved by conserving the homologous mixture of ABTS salt (20 mM) and potassium peroxydisulfate (70 mM) in a darksome condition for unremitting 24 hours. A specific 450 µL portion of the incubated solution was independently blended with 600 µL of different concentrations of trial specimens and maintained undisturbed for 10 minutes. Eventually, consequences were noted at 734 nm against the resembling concentrations of ascorbic acid [16].

In vitro antidiabetic scrutiny

Anti-α-amylase vetting: An admixture of the dissimilar trial samples (250 µL) and a homologous portion of phosphate buffer (0.02 M; pH 6.9) comprising α-amylase (500 µg/mL) were kept undisturbed at standard ambient temperature for 10 minutes. A 1 % solution of starch (250 µL) was complemented into all trial samples and retained at the same temperature for another 10 minutes. A 500 µL sugar estimation reagent (Dinitrosalicylic acid) was incorporated into all tubes; heated for a quarter-hour in the

thermostatic bath and homogenized with 5000 µL of distilled water. Experimental consequences were figured out at 540 nm. The resembling concentrations of acarbose were employed for reference consideration [17, 18].

Anti-α-glucosidase vetting: The appraisal of yellowish 4-hydroxy nitrobenzene developed from 4-nitrophenyl glucoside (p-NPG) is the base of the screened analysis. Within the experimented model, 50 µL of trial samples were homogenized with 100 µL of α-glucosidase (1 unit/mL) and kept undisturbed for a quarter-hour at ambient temperature. Reaction progression was attained with the addition of 50 µL solution of 3.0 mM p-nitrophenyl glucopyranoside and after a pause of 20 minutes, all samples were complemented with 2000 µL of 0.1 M soda ash. The out-turns of practical were figured out at 405nm and parallel concentrations of acarbose were oriented for reference analysis. [19].

In vitro anti-inflammatory scrutiny

Anti-proteinase vetting: Averting the proteolysis through inhibition of proteolytic biocatalyst is the appraisement criteria of the executed model. A composite of dissimilar trial concentrations (1000 µL), tris-hydrochloride buffer (1000 µL; pH 7.4), and trypsin (60 µg) were incubated in warm water for 5 minutes. A 1000 µL quantity of protein solution (0.3% w/v casein) was mingled and continued for incubation in warm water. After 20 minutes, each composite was combined with 70% hyperchloric acid (2000 µL), and submitted for centrifugation process at 3000 rpm. The upper surface from each centrifuged mixture was examined spectrophotometrically at 210 nm. The parallel concentrations of diclofenac were employed to accomplish the reference trials [20].

Anti-lipoxygenase vetting: Analysis of the transformation of linoleic acid to hydroperoxyl compounds is the basis of the applied anti-inflammatory appraisal. All specimens (50-250 µg/mL) were separately mingled with 250 µL of 2M boric acid buffer (pH 9); subsequently, 250 µL of lipoxygenase solution (20,000 U/mL) was supplemented and maintained at normal temperature for 5 minutes. A 0.6 mM linoleic acid suspension (1000 µL) was aggregated with all solutions, and an absorbance study was executed at 234 nm. The reference trials were discharged with the akin concentrations of diclofenac [21].

Figuring of % inhibition andIC50 value

In every individual study, figuring of % inhibition was done with *Formula 1* and the IC50 statistics were accomplished with the plot of concentration (x-axis) and % inhibition (y-axis).

Formula 1: % inhibition = $[(A_{con} - A_{sam})/A_{con}] \times 100$

(A_{con}: Absorbance from the control and A_{sam}: Absorbance from the sample)

RESULTS AND DISCUSSION

Phytochemical scrutiny: The chemical nature of aqueous and cow urine macerate was studied with definite qualitative tests, insinuating the habitation of disparate phytochemicals displayed in Table 1 including biologically active compounds like alkaloids, flavonoids, tannins, steroidal compounds, coumarins, and terpenoids. The cow urine extract also displayed a positive report for the existence of saponins.

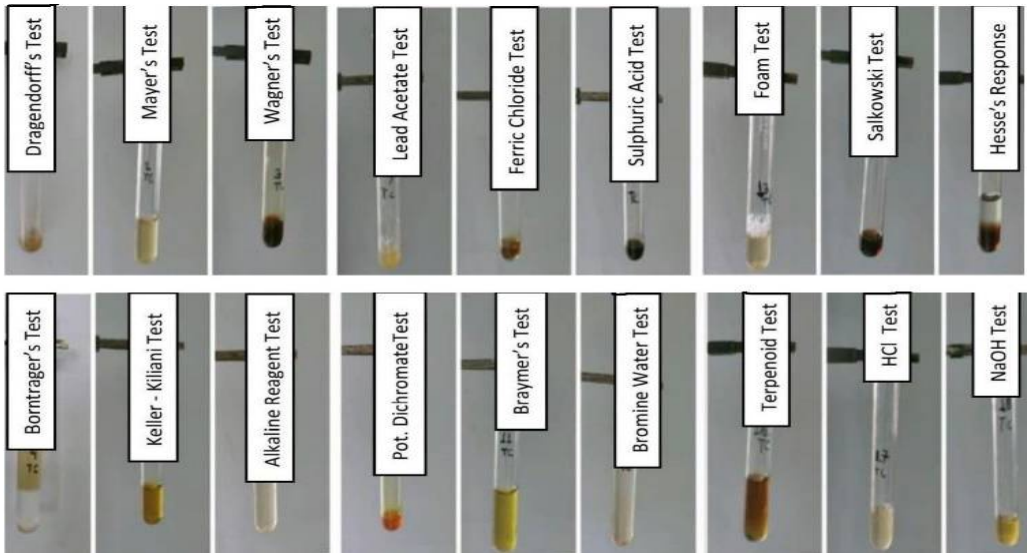

Fig. 2. Phytochemical scrutiny of cow urine extract of Trikatu Churna

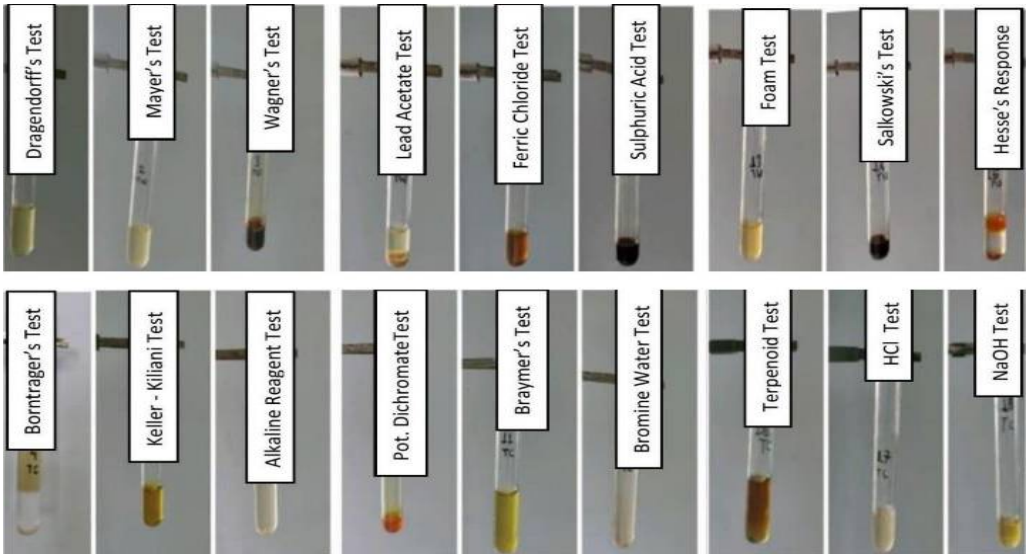

Fig. 3. Phytochemical scrutiny of aqueous extract of Trikatu Churna

Table 1.Phytochemical scrutiny of cow urine extract and aqueous extract of Trikatu churna

Phytochemicals	Test	Cow urine extract	Aqueous extract
Alkaloids	Mayer's Test	+	+
	Dragendroff's Test	+	-
	Wagner's Test	+	+
Cardiac Glycosides	Keller-Killiani Test	-	-
Flavonoids	Alkaline reagent Test	+	+
	Ferric Chloride test	-	-
	Lead acetate Test	+	+
	Sulphuric acid Test	-	-
Phenolic compounds and Tannins	Potassium dichromate Test	-	-
	Bromine water Test	+	+
	Braymer's Test	-	-
Saponins	Foam Test	+	-
Anthraquinones	Borntrager's Test	-	-
Phytosterols	Salkowski's Test	+	+
	Hesse's Response	-	+
Terpenoids	Chloroform-acid Test	+	+
Anthocyanins	HCl Test	-	-
Coumarins	NaOH Test	+	+

Antioxidative scrutiny: An experimental antioxidative model with DPPH reagent demonstrated that both extracts and ascorbic acid offer an increment in % inhibition with ascending order of concentration. The inhibition pattern of DPPH radicals suggests more competence of cow urine extract against oxidative damage than aqueous extract. The IC50 numerals computed from the regression plot (Fig. 4) were sequentially noted as 109.96, 164.04, and 12.51 µg/mL towards cow urine macerate, aqueous macerate, and ascorbic acid. The dynamic result of % inhibition from 50 to 250 µg of cow urine extract is the inkling of its efficient antioxidative competence. With the existence of antioxidant components, a violet tinge of the DPPH solution expresses the transformation to yellow color, which is an indication of the reduction of its nitrogenous free radicals. In the extant scrutiny, the reduction impact noticed with cow urine extract may be correlated with the proton donating adeptness of its bioactive components [5]. The finding of the ABTS scavenging assay pointed out that cow urine extract can prohibit the creation of radicals more prominently than aqueous extract. The IC_{50} numerals for cow urine extract, aqueous extract, and ascorbic acid were computed on the basis of the regression graph (Fig. 5) sequentially observed as 115.38, 151.16,and 71.97 µg/mL. The results of the ABTS scavenging assay manifested that % inhibition with cow urine extract was more potent while poor competency of % inhibition for the aqueous extract. The fading of the blue coloration of ABTS solution is an indication of the neutralization impact on oxidizing radicals. The

antiradical constituents in cow urine extract may donate more protons with boosting doses that express the antioxidative potentiality in ascending order [22].

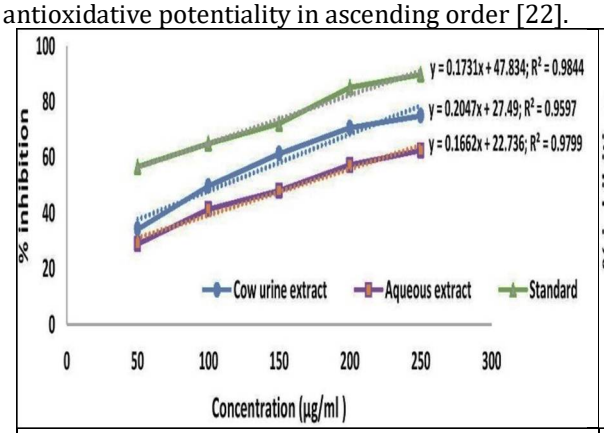

Fig. 4. DPPH radical scavenging assay

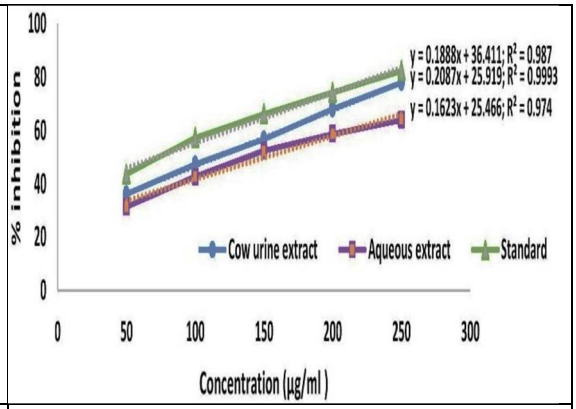

Fig. 5. ABTS radical scavenging assay

Antidiabetic scrutiny: The acquisition of inhibitory consequences against the hydrolytic activity of enzymes is the prominent model to appraise antidiabetic efficacy. The accomplished scrutiny against the α-amylase enzymes depicted in Fig. 6 expresses the captivating potentiality of cow urine macerate (IC50 statistic: 179.11 µg/mL). The same figure notified the weak performance of aqueous macerate (IC50 statistic: 232.82 µg/mL) while acarbose bestowed the supreme performance (IC50 statistic: 92.60 µg/mL). The scrutinized consequences against α-glucosidase are illustrated in Fig. 7. The outcomes of the assay denoted that, the cow urine extract observed through the IC50 statistic of 143.26 µg/mL displayed more effectuality than the aqueous extract (IC50 value: 215.54 µg/mL). Acarbose exhibited an intense inhibitory performance with the IC50 numeral of 32.88 µg/mL. The cow urine macerate causes a refrainment of hydrolytic enzymes in a concentration dependant mode is the essential mode to control the glucose level in the blood and it may provide a competent approach to control the hyperglycaemic situation. During the present study, the fundamental phytochemical scrutiny revealed the presence of numerous bioactive compounds including phenolic nature, and earlier research findings claim that phenolic compounds develop hydrogen bonding with these enzymes and thus obstructed their role in the hydrolysis of sugar molecules [23]. In hyperglycemic patients, the oxidation of glucose may engender the production of damaging radicals which may be responsible for different complications in the body. In such cases, the antiradical potentiality of phyto material may become a complementary therapy to sidestep these complications [24]. The enhanced antioxidative efficacy of cow urine extract may be the reason for the improvement in antihyperglycemic performance.

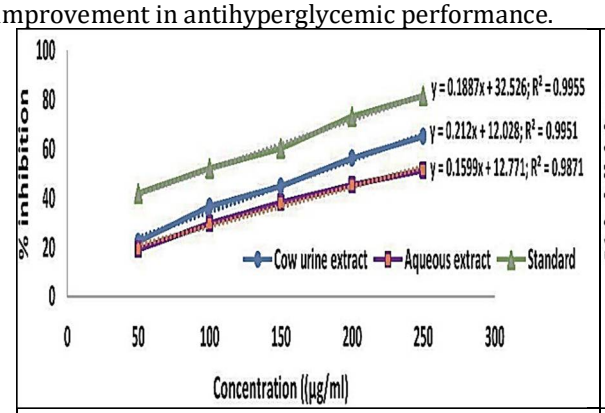

Fig. 6. α-Amylase inhibitory assay

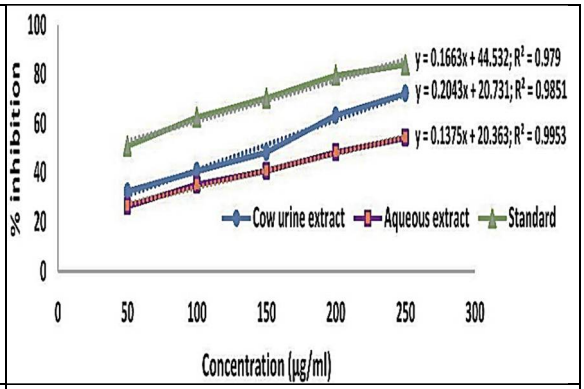

Fig. 7. α-Glucosidase inhibitory assay

Anti-inflammatory scrutiny: The inhibitory trials against proteinase and lipoxygenase are the essential mode to evaluate anti-inflammatory competency. The outcomes of the anti-proteinase assessment are illustrated in Fig. 8. The cow urine extract and aqueous extract were sequentially calculated with the IC50 numerals of 526.06 µg/mL and 266.12 µg/mL while the statistic for diclofenac presented 106.13 µg/mL as an IC50 numeral. The anti-proteinase assay reflected the poor result of IC50 for cow urine extract, denoting its insignificant efficacy than diclofenac. Inflammatory conditions are interrelated with different enzymes including Proteolytic enzymes and the refrainment of such enzymes can generate anti-inflammatory

prospects [25]. In the present anti-proteinase assay, the poor performance of cow urine extract might be linked with the urokinases recorded in cow urine [26]. The potentiality order during the anti-lipoxygenase assay (Fig. 9), was ascertained as standard drug > cow urine extract > aqueous extract. An experimented trial concentrations of cow urine macerate (50 to 250 µg/mL) presented the ascending order of % inhibition from 21.28 to 66.76% and disclosed 171.77 µg/mL as IC50 numeral. Diclofenac showed intense % inhibition with the IC50 numeral of 84.64 µg/mL while aqueous extract presented weak performance with the IC50 numeral of 228.60 µg/mL. The obtained results suggested that the anti-lipoxygenase action exhibited by cow urine extract may become the possibility for its anti-inflammatory potentiality. Lipoxygenase is noticed with an essential role in the production of inflammatory components from polyunsaturated fatty acids. The oxidative stress in hyperglycemia may stimulate lipoxygenase and may cause upregulation in the inflammatory response. [27]. In this case, the antioxidative potency of cow urine extract may become supportive of the anti-lipoxygenase efficacy. Prior research findings claimed that endogenous peptides reported in cow urine can express the binding with lipoxygenase and ceases their inflammatory role [28].

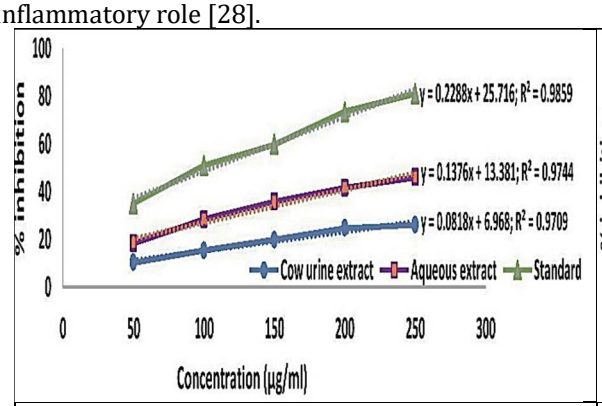

Fig. 8. Proteinase inhibitory assay

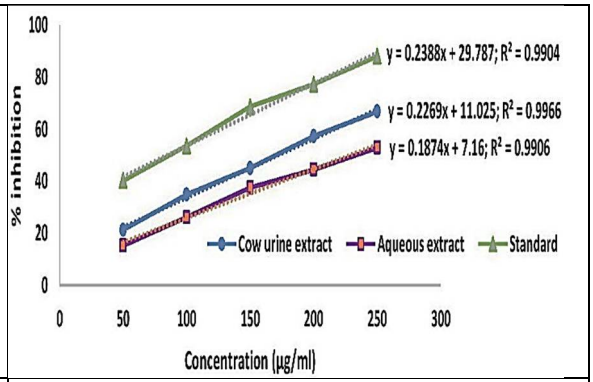

Fig. 9. Lipoxygenase inhibitory assay

CONCLUSION

The comprehensive results of the experimented work prompted that, conventional claims of herbal treatment in coalition with cow urine can generate a new therapeutic way for global healthcare. Furthermore, the presence of essential phytochemicals in cow urine extract of Trikatu churna may be responsible for the obtained outcomes that are necessary to recognize and link at the molecular level.

CONFLICT OF INTERESTS

No conflict of interest from the author's side.

AUTHOR CONTRIBUTIONS

Both authors contributed equallyduring research work and manuscript preparation.

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