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Evaluation of Hepatoprotective and Antioxidant Activity of Isolated Flavonoids Compounds from *Ougeinia oojeinensis* extracts

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ABSTRACT

The flavonoids present in plant grant different kinds of pharmacological action. It has been reported that the flavonoids assume boss job in assurance of harm liver. Subsequently we wanted to research the hepatoprotective and cancer prevention agent action of segregated flavonoids mixes from *Ougeinia oojeinensis* leaves extracts. The oil ether, ethanol and watery extracts were set up from leaves of *Ougeinia oojeinensis*, and screened for phytochemical investigation. The diverse flavonoid compound disengaged from ethanol separate, and further assessed their hepatoprotective and cancer prevention agent movement in CCl_4 prompted hepatotoxicity. The most extreme phytoconstituents alongside flavonoids were seen in ethanol extract of leaves of *Ougeinia oojeinensis*. The nine distinct divisions were isolated and dried from ethanol extracts of *Ougeinia oojeinensis*. The F8 and F9 demonstrate the nearness of flavonoids, tannins and phenolic mixes. The F8 and F9 indicates intense hepatoprotective action by essentially diminishing the CCl_4 prompted modification in SGOT, SGPT, ALP, ACP, all out bilirubin and direct bilirubin in blood. The cancer prevention agent movement of F8 and F9 affirmed on altogether expanding in dimension of catalase, glutathione peroxidase, superoxide dismutase compound, while lipid peroxidation was diminished, when contrasted with CCl_4 condition. The consequence of study recommends that the flavonoid compound F9 of *Ougeinia oojeinensis* showed most extreme hepatoprotective and cell reinforcement action thought about F8.

KEY WORDS: *Ougeinia oojeinensis*, Hepatoprotective, Antioxidant.

1. INTRODUCTION

Restorative plants and inferred drugs are generally utilized in conventional culture frameworks everywhere throughout the world. In most recent couple of decades, there has been an exponential development in the field of home grown drug and getting promoted in creating and created nations inferable from its regular inception and lesser sick impacts. It is reported that 80% of the word populace has confidence in conventional prescription, especially plant drugs for their essential medicinal services. Plants utilized for customary drug frameworks contain a wide scope of substances that can be utilized to treat endless, metabolic just as irresistible illnesses. Late pattern, notwithstanding, demonstrates that the disclosure rate of dynamic compound elements is declining. Normal results of higher plants may give another wellspring of hepatoprotective, antidiabetic, antiulcer, mitigating, immunomodulatory and so on specialists with potentially novel components of activity (Dubey, 2004).

The utilization of normal solutions for the treatment of liver illnesses has a long history, beginning with the Ayurvedic treatment, and reaching out to the Chinese, European and different frameworks of customary medications. The 21st century has seen a change in perspective towards helpful assessment of natural items in liver infection models via cautiously synergizing the qualities of the conventional frameworks of medication with that of the advanced idea of proof based restorative assessment, institutionalization and randomized fake treatment controlled clinical preliminaries to help clinical viability. An extensive number of plants and details have been professed to have hepatoprotective movement. About 160 phytoconstituents from 101 plants have been asserted by Pharmacopeia Foundation to have liver ensuring activity (Khan, 2016).

In India, in excess of 87 plants are utilized in 33 protected and restrictive multi-fixing plant definitions. Regardless of the huge advances made, no huge and safe Hepatoprotective specialists is accessible in present day therapeutics. In this way, due significance has been offered all inclusive to create plant-based hepatoprotective medications, compelling against an assortment of liver issue. A portion of the Hepatoprotective Herbs are *Allium hirtifolium, Allium sativum, Apium graveolens, Andrographis paniculata, Abutilon indicum, Borreria articularis, Berberis vulgaris, Boerhavia diffusa, Citrus microcarpa, Cichorium intybus, Cynara scolymus, Calendula officinalis, Eclipta alba, Foeniculum vulgare, Marrubium vulgare, Nigella sativa, Phyllanthus amarus, Prunus armeniaca, Prangos ferulacea, Salacia reticulata, Stachytarpheta indica, Taraxacum officinale, Tinospora Cordifolia, Wedelia calendulacea* and so on (Roy, 2014; Anonymous, 1997).

Ougeinia oojeinensis is a deciduous pretty tree. Plant extract showed antisplasmodic action, weak CNS depressant effect, anti-inflammatory, analgesic, antioxidant, anthelmintic, hepatoprotective activity, hypoglycemic, antidiabetic and wound healing activity. Traditionally it is used for the treatment of jaundice, diarrhoea, dysentery, urorrhagia, diabetes, verminosis, leprosy, leucoderma, haemorrhages, fevers, ulcers etc. (Gunasekaran, 2011; Samyal, 2014; Samyal, 2014; Velmurugan, 2013; Verma, 2012). Isolation of three isoflavanones, HPLC analysis of

genistein and two pentacyclic triterpene alcohol from the heart wood and bark part of the *Ougeinia dalbergioides* (Wankhade, 2015).

The hepatoprotective movement of *Ougeinia oojeinensis* leaves remove was experimentally announced. Be that as it may, no report has yet been distributed about the dynamic part in charge of hepatoprotective movement of *Ougeinia oojeinensis* leaves separate. The primary point of this investigation was to assess the hepatoprotective and cancer prevention agent movement of separated mixes *Ougeinia oojeinensis* extracts.

2 MATERIAL AND METHODS

Preparation of extracts: 500 gram of powdered of *Ougeinia oojeinensis* leaves coarsely powders were pressed independently in soxhlet extractor and extricated utilizing oil ether, ethanol and refined water progressively. The extract were then thought to dryness under diminished weight and controlled temperature, separately and they were safeguarded in an icebox. The ethanol and watery extracts were chosen for further investigation.

Phytochemical analysis: Qualitative chemical tests were performed to determine the presence of alkaloids, carbohydrates, cardiac glycosides, polyphenols, saponins, tannins and terpenoids (Purohit, 2014; Kenwat, 2014).

Isolation of Compounds from *Ougeinia oojeinensis* leaves extract: The *Ougeinia oojeinensis* separate was exposed to section chromatography utilizing silica gel (60-120 work estimate), and eluted with the accompanying dissolvable proportions of Hexane: Chloroform (CH), 100:0, 75:25, 50:50, 25:75, 0:100, at that point with 100:0, 75:25, 50:50, 25:75, 0:100; Chloroform (CH): Ethyl acetic acid derivation (EA), 100:0, 75:25, 50:50, 25:75, 0:100, at that point with 100:0, 75:25, 50:50, 25:75, 0:100 CH:Ethanol (Eth). The portions (25 ml) were gathered from the segment. The elute gathered were checked by thin layer chromatography (eluent: EA-Eth, 9:1 and 3:2) for homogeneity and the comparable division were pooled together. The nine distinct portions were gathered and dried. The portion F1 and F2 were containing waxy material; the parts F3, F4 and F7 were powder however amount was almost no (Chatterjee, 2014). The yield of part F5, F6, F8 and F9 were 210 mg, 170 mg, 380 mg and 280 mg, individually. The four divisions were additionally broke down for phytochemical screening to decide the idea of confined compound.

Hepatoprotective activity against CCl_4-induced hepatotoxicity: Group II to VII got CCl_4 (1ml/kg i.p.) with equivalent volume of olive oil (half v/v) to instigate intense poisonous quality, for two progressive days. Group I creatures filled in as ordinary group and controlled saline and typical sustenance. Group II creatures were kept up as CCl_4 group, while Groups III and IV creatures were dealt with orally for seven days with suspension of chloroform remove at the portion of 200 mg/kg and 400 mg/kg, separately. Group V and VI creatures were dealt with orally for seven days with suspension of ethanol separate at the portion of 200 mg/kg and 400 mg/kg individually. Group VII creatures were treated with standard medication silymarin (25 mg/kg). After the medication treatment every one of the creatures were yielded by cervical disengagement. Blood was gathered from the carotid course and was permitted to clump for 45 min at room temperature; serum was isolated by centrifugation at 2500 rpm for 15 min, utilized for the estimation of different biochemical parameters.

Serum analysis: Serum separated by centrifugation were used to determined serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), serum acid phosphatase (ACP) and serum bilirubin (Sharma, 2017).

Liver homogenates preparation and biochemical estimation (*In vivo* antioxidant activity): Solidified liver examples were homogenized (with a Potter-Elvehjem homogenizer) in a Tris-HCl support or in a phosphate cradle arrangement (PBS) to give a 20% homogenate. To assess dimensions of lipid peroxidation, the homogenate was centrifuged at 1700 rpm/min for 10 min at 4°C. To evaluate catalase movement, the homogenate was centrifuged at 3500 rpm/min for 15 min at 4°C and after that weakened up to 5%. Further on, the supernatant was again centrifuged either at 10,000 rpm/min for 1 min and weakened to 2% for estimation of glutathione peroxidase movement or at 30,000 rpm/min for 10 min before extraction of tissue superoxide dismutase action with 20% ethanol (Gupta, 2012).

Measurable examination: The outcomes are communicated as mean ± SEM of six autonomous investigations. Factual criticalness between the groups was assessed by single direction investigation of difference (ANOVA) trailed by Dunet's test. A $P < 0.05$ esteem was considered as measurably huge.

3. RESULTS AND DISCUSSION

In the present study, *Ougeinia oojeinensis* were selected for isolation of active constituents from extract and evaluate hepatoprotective activity of isolated compounds.

Phytochemical screening of *Ougeinia oojeinensis*: Starter phytochemical examinations of the extract of leaves of *Ougeinia oojeinensis* uncovered the nearness of flavonoids, tannins, phenolic mixes, alkaloids, glycosides, fats and starches. The subtleties are exhibited in table.1.

From the consequence of phytochemical screening, the oil ether extracts of leaves of *Ougeinia oojeinensis* showed the nearness of fats and oils. Alkaloids, glycosides, starches, flavonoids, tannins, proteins and polyphenol were found in ethanol extract of leaves of *Ougeinia oojeinensis*. Likewise glycosides, sugars, flavonoids, tannins and

polyphenol were existing in watery extract of leaves of *Ougeinia oojeinensis*. The most extreme phytoconstituents alongside flavonoids were seen in ethanol extract of leaves of *Ougeinia oojeinensis* (Table.1). Henceforth ethanol extract of *Ougeinia oojeinensis* was chosen for further division of compound as this extracts uncovered the nearness of flavonoids and phenolic mixes.

Table.1. Phytochemicals present in leaves of *Ougeinia oojeinensis* extracts

Phytoconstituent	Pet. Ether	Ethanol	Aqueous
Alkaloids	-	+	-
Glycosides	-	+	+
Carbohydrates	-	+	+
Tannins and Phenolic compound	-	+	+
Flavonoids	-	+	+
Steroid test	-	-	-
Protein	-	+	-
Fat and oil test	+	-	-

+ = Present, – = Absent

Isolation of compound from ethanol extract of *Ougeinia oojeinensis*: The nine unique parts were gathered and dried from ethanol extracts of *Ougeinia oojeinensis*. The division F1 and F2 were containing waxy material; the portions F3, F4 and F7 were powder however amount was practically nothing. The yield of part F5, F6, F8 and F9 were 210 mg, 170 mg, 380 mg and 280 mg, separately. The four portions were additionally dissected for phytochemical screening to decide the idea of disengaged compound.

Starter phytochemical examination of detached portion of ethanol extracts of *Ougeinia oojeinensis.*

The phytochemical examination of F5 of *Ougeinia oojeinensis* uncovered the nearness of alkaloids and sugars. The F6 showed the nearness of alkaloids, glycosides and starches. The F8 and F9 show the nearness of flavonoids, tannins and phenolic mixes and flavonoids (Table.2).

Table.2. Preliminary phytochemical analysis of isolated fraction of ethanol extract of *Ougeinia oojeinensis*

Phytoconstituents	F5	F6	F8	F9
Alkaloids	+	+	-	-
Glycosides	-	+	-	-
Carbohydrates	+	+	-	-
Tannins & Phenolic compounds	-	-	+	+
Flavonoids	-	-	+	+
Steroids	-	-	-	-
Proteins and Amino acids	-	-	-	-
Fixed Oils and Fats	-	-	-	-

(+) Present, (-) Absent

The auxiliary metabolites bestow different pharmacological exercises in particular enemy of tumor, pain relieving, hostile to joint pain, antidiabetic, hepatoprotective and so on. The parts got from the ethanol extracts of *Ougeinia oojeinensis* showed different kinds of auxiliary metabolites. Numerous examinations have demonstrated that assortments of flavonoid atoms have hepatoprotective action. Therefore, it might be significant to constantly assess the hepatoprotective action of flavonoids, for setting up hepatoprotective systems, as well as for building up another class of hepatoprotective operators. The F8 and F9 containing polyphenol and flavonoids compound and these natural substances give boss job in hepatoprotective action. Thus this outcome underpins us to assess the hepatoprotective action of the F8 and F9.

Hepatoprotective action: The *in vitro* investigations of the F8 and F9 confined from *Ougeinia oojeinensis* leaves separates shows the nearness of flavonoids and polyphenol. Along these lines hepatoprotective movement were performed with F8 and F9.

Hepatoprotective movement of disengaged flavonoids was resolved in $CC1_4$ instigated hepatotoxicity display. In the present investigations, rodents treated with CCl_4, created critical liver harm as saw from the raised serum dimensions of hepato-explicit catalysts just as extreme change in other biochemical parameters. Estimations of the biochemical parameters were altogether expanded in the CCl_4 treated rodents (Table.3).

Table.3. Effect of isolated flavonoid component of *Ougeinia oojeinensis* in CCl_4 induced liver toxicity in rats

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	ACP (U/L)	Bilirubin (mg/100 ml of blood	
					Direct (mg/dl)	Total (mg/dl)
Normal Control CMC (10ml/kg)	71.6±8.1	122.4±4.8	165.1±5.6	132.4±2.4	0.32±0.2	0.69±0.4
CCl_4 (1ml/kg i.p.)	215.2[a]±5.3	354.7[a]±2.6	343.9[a]±3.7	401.3[a]±5.8	2.51[a]±0.5	3.87[a]±0.1
F8 (50 mg/kg)	135.3*±2.7	198.2*±6.3	251.7*±4.8	221.5*±4.1	1.32*±0.1	2.31*±0.4
F8 (100 mg/kg)	86.1*±4.1	152.9*±3.7	186.7*±6.2	163.2*±7.0	0.42*±0.2	0.86*±0.5
F9 (50 mg/kg)	112.6*±2.5	165.1*±5.6	210.2*±3.4	192.6*±4.2	1.02*±0.1	1.95*±0.4
F9 (100 mg/kg)	76.2*±6.3	131.7*±4.1	156.4*±6.3	125.1*±5.8	0.33*±0.5	0.62*±0.3
Silymarin (25 mg/kg oral)	70.9*±4.3	119.4*±6.2	159.2*±3.7	128.7*±5.1	0.31*±0.3	0.68*±0.2

Values are expressed as mean ± SEM, n = 6 in each group. [a]P<0.05 when compared with normal control group, *P<0.05 when compared with CCl_4 treated group.

Treatment with the two dosages of F8 and F9 separated from of *Ougeinia oojeinensis* diminished the CCl_4 incited adjustment in SGOT, SGPT, ALP, ACP, complete bilirubin and direct bilirubin in blood. It was discovered that the test tests offer security against poison as confirm by exceptional decrease in every single biochemical parameter (P<0.05). It likewise uncovered that diminished biochemical parameter dimensions of both separated compound are less contrasted with standard medication. The part F9 displayed most extreme hepatoprotective action contrasted and F8.

***In-vivo* antioxidant activity:** Results from the cancer prevention agent assessment are appeared table.4. The organization of the F8 and F9 disconnected from of *Ougeinia oojeinensis* balanced the CCl_4-actuated free extreme action, which looks like that of silymarin. Grass, GPx and CAT catalyst levels are measurably noteworthy expanded, while lipid peroxidation is diminished, when contrasted with CCl_4 condition (P < 0.05). These outcomes recommend that the F8 and F9 segregated from of *Ougeinia oojeinensis* shows a cancer prevention agent movement. The part F9 displayed most extreme cancer prevention agent action contrasted with F8.

Table.4. Antioxidant activity of isolated flavonoid component of *Ougeinia oojeinensis* in CCl_4 induced liver damage in rats

Treatment	Catalase (mg liver protein) $^{-1}$	Superoxide dismutase (mg liver protein) $^{-1}$	Glutathione Peroxide (mg liver protein) $^{-1}$	TBA (mg/liver protein)
Normal Control CMC (10ml/kg)	256.2±5.3	81.5±3.2	1.21±0.5	1.56±0.2
CCl_4 (1ml/kg i.p.)	112.7±4.2[a]	22.1±8.4[a]	0.32±0.3[a]	4.21±0.8[a]
F8 (50 mg/kg)	185.1±6.1*	55.8±3.7	0.71±0.7	3.15±0.2*
F8 (100 mg/kg)	231.8±3.8*	79.1±2.8*	1.15±0.9*	1.82±0.4*
F9 (50 mg/kg)	198.7±4.9*	61.5±5.4*	0.86±0.7	2.72±0.4*
F9 (100 mg/kg)	251.6±2.7*	85.1±4.6*	1.32±0.6*	1.69±0.5*
Silymarin (25 mg/kg)	263.1±6.1*	82.3±3.1*	1.26±0.4*	1.47±0.2*

Values are expressed as mean ± SEM, n = 6 in each group. [a]P<0.05 when compared with normal control group, *P<0.05 when compared with CCl_4 treated group.

Liver take part in a few metabolic exercises, and so as to satisfy this job, discharge a wide assortment of catalysts. Liver can be harmed by numerous toxicants, just as by synthetic compounds or medications. In our model, CCl_4 fills in as a toxicant. CCl_4-related hepatotoxicity is related with rise in chemical dimensions, which might be ascribed to the age of trichloromethyl free radical amid digestion by the hepatic microsomes, which thusly start lipid peroxidation. Hepatocellular rot diminishes SOD, CAT and GPx exercises, and the expansion of such exercises into basal qualities, is a reasonable sign of plasma layer adjustment and tissue fix also. Such an impact is in concurrence with the view that catalyst exercises are reestablished into typical conditions and recuperating of the hepatic parenchyma, just as hepatocyte recovery, are watched. Turf, CAT, and GPx comprise a protein guard system against oxidative harm. Under CCl4 conditions such compound exercises are diminished, yet under plant-treated conditions, a huge increment in their exercises is watched, which may fill in as a biochemical technique to decrease lipid peroxidation (Lin, 2000). The examination uncovered that the F8 and F9 disconnected from of *Ougeinia oojeinensis* under assessment, at the two investigations dosages, demonstrated a hepatoprotective action against CCl_4-incited

liver harm. The hepatoprotective movement of F8 and F9 secluded from *Ougeinia oojeinensis* proposed their cancer prevention agent action due to polyphenol and flavonoid nature.

4. CONCLUSION

The flavonoid F8 and F9 segregated from of *Ougeinia oojeinensis* have solid hepatoxic prevention agent action and may present a gainful impact against oxidative pressure. In view of the consequences of in vivo test, the hepatoprotective activity of F8 and F9 secluded from of *Ougeinia oojeinensis* was because of its cancer prevention agent property. From discoveries it has been reasoned that the F9 of *Ougeinia oojeinensis* displayed greatest hepatoprotective and cell reinforcement movement thought about F8.

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