RESEARCH ARTICLE

Phytochemical Analysis and an *In-vitro* Antioxidant Activity of *Gokshuradi Churna*, An Ayurvedic Polyherbal Formulation

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Received: 29/08/2023 **Accepted:** 13/12/2023 **Published:** 15/02/2024

Abstract

Introduction: The branch of traditional medicine constitutes Ayurveda, Siddha, Unani and Homeopathy. The knowledge of these traditional systems of medicine with the perspective of safety, efficacy, and quality will helps to the traditional legacy and also to rationalize the use of natural products in healthcare as well.

Aim and Objective: The aim and objective of this study was to evaluate an *in vitro* antioxidant activity and quantification of phytochemicals of polyherbal drug, *Gokshuradi churna (G. churna)*.

Methods: An aqueous extract of *G. churna* of 10mg/mL concentration was used for the estimations of total phenols, flavanoids, saponins and tannins. Free radical scavenging activity in terms of DPPH, superoxide and nitric oxide scavenging activity of *G. churna* was carried out by the standard methods.

Results: In this study the phytoconstituents such as flavonoids (69.11±5.31 mg/g quercetin equivalent), phenols (56.39 ± 4.07 mg/g gallic acid equivalent), saponin (73.43 ± 3.41 mg/g diosgenin equivalent) and tannin (58.11±1.41 mg/g tannic acid equivalent) were noticed in *G. churna*. Among which saponin and flavonoids showed highest content of 73.43 mg/g and 69.11 mg/g respectively in *G. churna.* An antioxidant activity was evaluated by determining the DPPH radical scavenging concentration (IC_{50}: 79.47 µg/mL; Standard IC_{50}: 59.84 µg/mL), superoxide radical concentration (IC_{50}: 125.12 µg/mL; Standard IC_{50}: 91.57 µg/mL) and nitric oxide radical concentration (IC_{50}: 244.85 µg/mL; Standard IC_{50}: 264.41 µg/mL) under *in vitro* conditions.

Conclusion: The present study established that the drug showed an effective IC_{50} concentration for nitric oxide radical scavenging activity. It also scavenges DPPH and superoxide radical scavenging activity. The antioxidant activity of the drug may be due to the polyherbal ingredients present in it. The antioxidant activity in terms of scavenging nitric oxide radical was significantly higher than the standard ascorbic acid and also its activity was concentration-dependent.

Keywords: Gokshuradi churna, DPPH radical scavenging, Nitric oxide radical, Superoxide radical, Secondary metabolites

Journal of Applied Pharmaceutical Sciences and Research, (2023); DOI: 10.31069/japsr.v6i4.02

Introduction

The branch of traditional medicine constitutes different components such as Ayurveda, Siddha, Unani and Homeopathy. The knowledge of these traditional systems of medicine with the perspective of safety, efficacy, and quality will helps to the traditional legacy and also to rationalize the use of natural products in healthcare as well.[1, 2] In the present study, an attempt was made to evaluate the antioxidant potential of *G. churna,* an ayurvedic polyherbal formulation made up of nine ingredients which are used in the treatment of heart ailments[3] and an excellent remedy for sexual performance.[4] The ingredients of *G. churna* are *Gokshura* (*Tribulus terrestris* L.), *Tankana* –Borax (Sodium Pyroborate), *Soraka* (Potassium chloride), *Punarnava* (*Boerhavia diffusa* L.), *Haritaki* (*Terminalia chebula* Retz.), *Yavakshara* (alkaline formulation prepared using dried whole plant of *Hordeum vulgare* L.), *Svarijiksara* (salt of sodium bicarbonate), *Kankola* (*Piper cubeba*. L.), *Revalchini* (Chinese rhubarb). *G. churna* is prepared by mixing all these ingredients in equal parts. Also, studies suggested that *G. churna* has both revitalising and rejuvenating property. The anti-inflammatory and diuretic properties of this formulation make it extremely useful in improving kidney functions and treating a number of genitourinary problems like urinary tract infection, urinary distension, urinary calculi, dysuria, difficulty in micturition, treating osteoarthritis and gout.[5-9] *Gokshura* has various names like *Goksuraka*, *Trikanata*, *Gokhru*, *Gokhuri*, *Gokshra*, Devil's thorn and Puncture vine. It also helps in building muscle mass and improving brain activity.[10] It has earned the name trikanta or puncture vine since the fruit has sharp thorns along its surface which is hard enough to even puncture a cycle tyre. *Gokshura* obtained from the dried fruits of the *Gokshura* tree which (*Tribulus terrestris)* which is a perennial plant that thrives in both cool and hot temperatures. The plant has active constituents like alkaloids and phytosterols which helps in anti-diuretic and antioxidant property of these plants.[11]

Polyphenols protect cell constituents against destructive oxidative damage and limits the risk of various degenerative

diseases associated with oxidative stress by acting as potent free radical scavengers. The polyphenol antioxidant activity is due to the chemical structure and ability to donate/accept electrons, thereby delocalising the unpaired electron within the aromatic structure.[12] They play an important role in aging and age-related pathogenesis like cancer, hypertension, atherogenesis, Alzheimer's and Parkinson's disease.[3]

Materials and Method

G. churna was procured from IMPCOPS, Chennai. An aqueous extract of *G. churna* of 10 mg/mL concentration was used for the estimations of total phenols, flavanoids, saponins and tannins.

Estimation of Total Phenols

Total phenolic content of *G. churna* was determined by standard procedure using gallic acid as a reference standard.[13,14] A volume of 0.05 mL of the *churna* preparation was mixed with 2.0 mL of 10N Folin-Ciocalteu reagent and neutralized with 4.0 mL of 7.5% w/v sodium carbonate solution. The reaction mixture was incubated at room temperature for 30 minutes with intermittent shaking for color development. The absorbance was measured at 765 nm using UV-Visible spectrophotometer (Thermo Scientific, Evolution 201). The total phenolic contents were determined using standard gallic acid. The total phenolic contents were expressed as mg/g gallic acid equivalent (GAE/g) of churna.

Total Phenols in mg/g of gallic acid equivalent: C = C1 x V/m

(C-Total phenolic content in mg/g of gallic acid; C1-Concentration of gallic acid derived from the calibration curve in mg/ml; V-Volume of sample in ml; m-Mass of the drug in g)

Estimation of Total Flavanoids

Total flavonoid content was measured from *G. churna* by standard procedure using quercetin as a standard[15,16] with the aluminium chloride colorimetric assay. 1.0 mL of aliquots and 1.0 mL standard quercetin solution was taken into a series of test tubes and 4.0 mL of deionised water and 0.3 mL of 5% sodium nitrite solution was added. After 5 minutes, 0.3 mL of 10 % aluminum chloride was added, at 6th minute, 2.0 mL of 1.0M sodium hydroxide was added. Finally, volume was made up to 10.0 mL with distilled water and mixed well. Orange yellowish color developed was measured at 510 nm using UV-Visible spectrophotometer (Thermo Scientific, Evolution 201).

Total Flavonoids in mg/g of quercetin equivalent: C = C1 x V/m

(C-Total flavonoids in mg/g of quercetin; C1-Concentration of quercetin derived from the calibration curve in mg/ml; V-Volume of sample in ml; m-Mass of the drug in g)

Estimation of Total Saponins

Total Saponins from *G. churna* was determined using diosgenin as a reference standard.[17] Standard diosgenin solution was prepared by dissolving 10 mg in 16 mL of methanol and 4.0 mL deionised water. To the aliquots, 0.25 ml of 8% vanillin reagent and 2.5 ml of 72% v/v sulphuric acid was added slowly along the sides of test tube. The solutions were mixed well and the tubes were incubated at 60°C in a water bath for 10 minutes. The tubes were cooled in ice cold water bath for 3-4 minutes and the absorbance was measured at 544 nm against the reagent blank in UV-Visible spectrophotometer (Thermo Scientific, Evolution 201). Results were expressed as mg of diosgenin equivalent/g (mg DE/g) of *churna*.

Total Saponin in mg/g of diosgenin equivalent: C = C1 x V/m

(C-Total saponin in mg/g of diosgenin; C1-Concentration of diosgenin derived from the calibration curve in mg/ml; V-Volume of sample in ml; m-Mass of the drug in g)

Estimation of Total Tannins

Total Tannins in *churna* was determined by Folin–Denis method.[18] To 0.05 mL of *churna* extract, added 1.0 mL of deionised water and then mixed with 0.5 ml of Folin–Denis reagent. The reaction mixture was alkalinized by the addition of 1.0 mL of 15% (w/v) sodium carbonate solution and kept in dark for 30 minutes at room temperature. The absorbance was measured at 700 nm using UV-Visible spectrophotometer (Thermo Scientific, Evolution 201). The concentration of tannins in the extract was determined using pure tannic acid as standard. Results were expressed as mg of Tannic acid equivalent/g (mg TE/g) of *churna*.

Total tannins in mg/g of tannic acid equivalent: C = C1 x V/m

(C-Total tannin in mg/g of tannic acid; C1-Concentration of tannic acid derived from the calibration curve in mg/ml; V-Volume of sample in ml; m-Mass of the drug in g)

Determination of DPPH radical scavenging activity

Free radical scavenging activity of *G. churna* was carried out by the standard method.[19] Briefly, 0.1 mL of various concentrations of standard and *G. churna* from 20 -150 µg/mL was taken into a series of test tubes and added 0.1 mL DPPH and incubated at 37°C for 30 minutes. An equal amount of methanol and DPPH was served as control. Absorbance was measured at 517 nm using UV-Visible spectrophotometer (Thermo Scientific, Evolution 201). The percentage of radical scavenging activity was calculated as per the formula given below:

Calculation

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test} \times 100}{\text{Absorbance of control}}$$

Determination of Nitric oxide scavenging activity

Nitric oxide scavenging activity of *G. churna* was assessed using standard procedure.[20] In this method, 2.0 mL of sodium nitroprusside prepared in 0.5 mL of phosphate buffered

saline and mixed with various concentrations (50-500 µg/mL) of *G. churna* and the mixture was incubated at 25°C for 150 minutes. Added 0.5 mL of Griess reagent to the mixture and this was incubated at room temperature for 30 minutes. Standard ascorbic acid was treated as above and absorbance was measured at 540 nm in UV-Visible spectrophotometer (Thermo Scientific Evolution 201). The amount of nitric oxide radical inhibited by the drug was calculated using the equation.

Calculation

Nitric oxide radical scavenging activity (%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance of test} \times 100}{\text{Absorbance of control}}$$

Determination of Superoxide anion radical scavenging activity

Superoxide anion radical scavenging activity of *G. churna* was evaluated with slight modification.[21] Briefly, 0.2 mL of nitro blue tetrazolium (0.08mM), 0.4mL of NADH (0.25mM) and 0.2mL of phenazinemetho sulfate solution to the mixture. Various sample concentrations (25-150 µg/mL) and quercetin standard were taken. The absorbance was read at 560 nm after being incubated at room temperature for 10 minutes in dark. The superoxide scavenging ability was calculated using the following formula.

Calculation

Superoxide anion radical scavenging activity (%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance of test} \times 100}{\text{Absorbance of control}}$$

Results

The results of phytoconstituents are given in Table 1. The phytochemicals such as flavonoids (69.11 mg/g quercetin equivalent), phenols (56.39 mg/g gallic acid equivalent), saponnin (73.43 mg/g diosgenin equivalent) and tannin (58.10 mg/g tannic acid equivalent) were noticed in *G. churna*. Among which saponin and flavonoids showed highest content of 73.43 and 68.76 respectively in *G. churna*.

DPPH radical scavenging activity

DPPH radical scavenging activity is shown in Figure 1 & Table 2. *G. churna* showed the highest percentage of DPPH scavenging activity at a concentration of 150 µg/mL. At this concentration, the drug scavenged 78.8 % and an IC_{50} concentration of 79.47 µg/mL and the ascorbic acid standard showed the highest of 87.6% inhibition at the concentration of 150 µg/mL and IC_{50} value of 59.84 µg/mL. *G. churna* showed 1.33-fold decreased activity when compared to standard ascorbic acid.

Table 1: Total Flavonoids, Total phenols, Saponin and Tannin content of *G. churna*

S. No.	*Parameters*	*Results*
1	Total Flavonoids (mg/g quercetin equivalent)	69.11 ± 5.31
2	Total Phenols (mg/g gallic acid equivalent)	56.39 ± 4.07
3	Saponnin (mg/g diosgenin equivalent)	73.43 ± 3.41
4	Tannin (mg/g Tannic acid equivalent)	58.11 ± 1.41

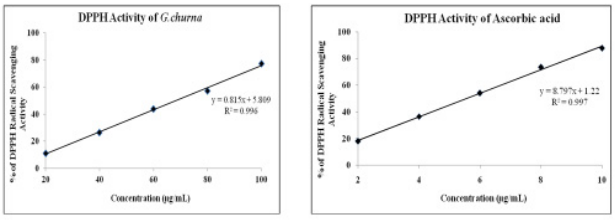

Figure 1: DPPH radical scavenging activity of *G. churna*

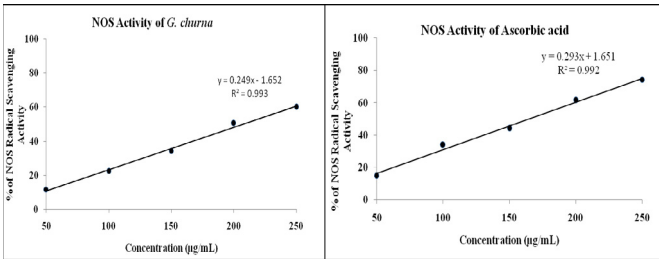

Figure 2: Nitric oxide radical scavenging activity of *G. churna*

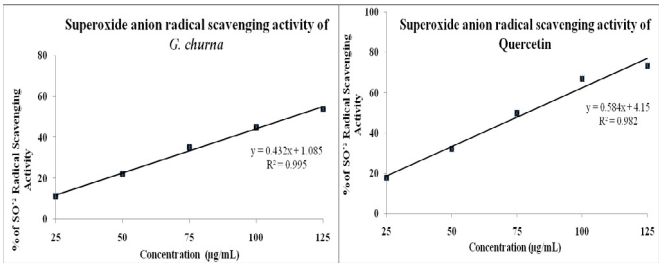

Figure 3: Superoxide anion radical scavenging activity of *G. churna*

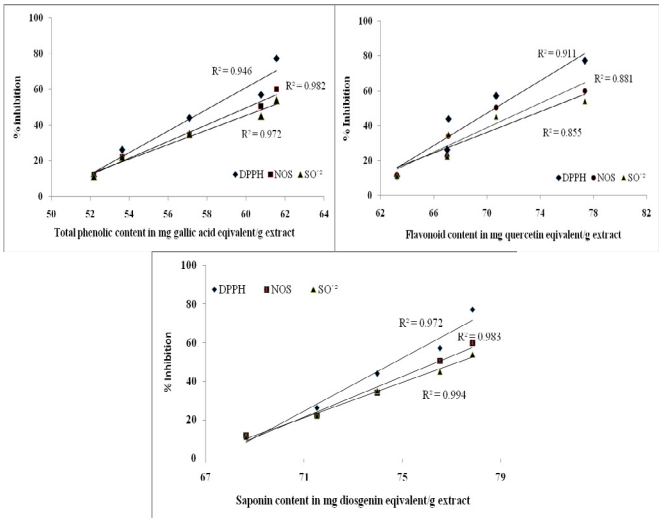

Figure 4: Comparison between phytoconstituents and antioxidant activity of ***G. churna***

Nitric oxide (NO) radical scavenging activity

Nitric oxide radical scavenging activity is shown in Figure 2 & Table 2. *G. churna* showed the highest percentage of nitric oxide radical scavenging activity at a concentration of 500

Table 2: *In vitro* Antioxidant activity of *G. churna*

Parameters	*G. churna (µg/mL)*	*Maximum Inhibition (%)*	*Standard IC50 (µg/mL)*	*Maximum Inhibition (%)*
DPPH radical scavenging activity (IC_{50})	79.47	78.8	59.84	87.6
Nitric oxide radical scavenging activity (IC_{50})	244.85	70.2	264.41	79.6
Superoxide anion radical scavenging activity (IC_{50})	125.12	60.6	91.57	80.0

µg/ml. At this concentration, the drug scavenged 70.2 % and an IC_{50} value of 244.85 µg/mL and the ascorbic acid standard showed highest percent of 79.6 at the concentration of 500 µg/mL and IC_{50} value of 264.41 µg/mL.

Superoxide ($SO^{\cdot}$) anion radical scavenging activity

Super oxide anion radical scavenging activity is shown in Figure 3 & Table 2. *G. churna* showed the highest activity at a concentration of 150 µg/mL which scavenged 60.6% of superoxide radicals and an IC_{50} value of 125.12 µg/mL. The highest activity of 150 µg/mL for standard quercetin showed 80.0% and IC_{50} value of 91.57 µg/mL. *G. churna* showed 1.32 fold decreased in super oxide anion radical scavenging activity when compared to standard quercetin.

Discussion

The *G. churna* have differential role in curing diseases as it has anti-oxidant, anti-inflammatory and anti-arthritic properties.[22] As a whole *G. churna* is a potent remedy for treating urinary disorders like urinary incontinence. The formulation is extremely effective in treating various heart ailments due to its strong antioxidative nature and hence diminishes the risk of heart attacks, strokes etc. Owing to the anti-inflammatory and analgesic properties of *Gokshura*, this powdered formulation plays a key role in relieving pain and inflammation and hence the *churna* can be widely used for alleviating pain in case of rheumatoid arthritis and osteoarthritis. The potent antioxidants present in *G. churna* improve the memory attention, concentration, calmness and alertness of an individual.

The DPPH radical scavenging activity of *G. churna* might be due to the ingredients present in it. Nitric oxide is diffusible free radical that plays several roles as an effector molecule in biological systems, including neuronal communication, vasodilatation, antimicrobial and antitumor activities.[23] Moreover, in pathological conditions, nitric oxide reacts with superoxide anion and forms potentially cytotoxic molecules, such as peroxynitrile which directly induces toxic reactions, as well as SH-group oxidation, lipid peroxidation, protein tyrosine nitration and DNA modifications. *G. churna* has noticeable activity against nitric oxide radicals. Superoxide plays a vital role in the formation of hydroxyl radical or singlet oxygen in living organisms. The free radicals formed are highly reactive and binds with lipids, DNA and proteins of the cell and cause deleterious effects. The activity may be related to the presence of phenols and flavonoids in the plants. *G. churna* scavenges superoxide radicals in a dose-dependent manner. *T. terrestris is* used in wide variety of conditions like infertility, urinary disorders, inflammation and ascites etc. The potent anti-oxidant property of this plant aided its use in many disease conditions. According to the previous study on *Gokshura* (*T. terrestris*) exhibited strong free radical scavenging and antioxidant activity compared to the control.[24] Chemical profiling of *T. terrestris* revealed that the presence of di-*p*-coumaroylquinic acid derivatives could be the reason for potent anti-oxidant activity which was studied through DPPH assay.[25] Our study results (Figure 4) showed the presence of significant amount of phenolic content (56.39 ± 4.07 mg/g gallic acid equivalents) and flavonoids (69.11 ± 5.31 mg/g quercetin equivalents) could also contributed to the anti-oxidant activity of this formulation. These results are consistent with the similar study by Dakshayani *et al.*[26]

A study by Patel showed that *B. diffusa*, one of the ingredients of *G. churna* found to be potent scavenger of superoxide, hydroxyl, nitrous oxide and DPPH radicals.[27] Beegum *et al*., studied the anti-oxidant potential of *B. diffusa* in different extracts and found that these extracts have potent antioxidant activity against free radicals, prevent damage of biomolecules and protect against oxidative damage.[28] *Haritaki* (*T. chebula*) also the ingredient of *G. churna* showed high content of phenols which attributed to the potent anti-oxidant property of this plant which justifies its use in wide variety of conditions. An *in vitro* and *in vivo* anti-oxidant activity may be due to the presence of gallic acid and ellagic acid present in *T. Chebula*.[29] There are studies which proved the anti-oxidant activity of *Kankola* (*Piper cubeba* L.) and *Revalchini* (*Chinese rhubarb*) which demonstrated the potent anti-oxidant activity of these plants.[30,31]

Conclusion

The antioxidant activity of *G. churna* in terms of nitric oxide radical is significantly higher than the standard ascorbic acid and also its activity was concentration dependent. Each plant ingredient of *G. churna* has potent anti-oxidant activity which contributed to the overall effective anti-oxidant activity of the formulation. The extensive health benefits of the various herbs used in this formulation also increases its therapeutic efficacy. Further *in vivo* studies may be needed to support this *in vitro* antioxidant activity.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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How to cite this article: Chitra S, Srilatha K, Ilavarasan R. Phytochemical analysis and an *In-vitro* antioxidant activity of *Gokshuradi churna*, An Ayurvedic polyherbal formulation. Journal of Applied Pharmaceutical Sciences and Research. 2023; 6(4):6-11 Doi : 10.31069/japsr.v6i4.02