Original Research Article

Phytochemical, UV-Visible and FTIR Assessment Along With In vitro Antioxidant Activity of Methanolic Extract of Tephrosia Purpuria Linn Root

ABSTRACT

Aims: The original phytochemical, UV-Visible, and FTIR Spectral estimation of Tephrosia purpuria root was the subject of this study. Natural phytoconstituents were all found in methanolic extracts from the root of Tephrosia purpuria.
Study design: Experimental research work.
Methodology: Furthermore, using UV Visible spectrophotometer equipment, the extract was scanned in the range of 380 to 900 nm, and the characteristic peaks were identified.
Results: The UV-VIS data indicated peaks at 382.70, 413.68, 536.18, 610.37, and 664.61 nm, with absorption values of 2.7930, 2.5932, 0.3114, 0.4185, and 1.5966 respectively. The presence of Natural phytoconstituents is confirmed by FTIR spectra. The findings confirm that this plant has key bioactive elements that are beneficial to our health, indicating that more research is needed.
Conclusion: Natural phytoconstituents were all found in methanolic extracts from the root of Tephrosia purpuria.

Keywords: Natural phytoconstituents, UV-Visible, FTIR, Tephrosia purpuria, Bioactive elements

1. INTRODUCTION

The discovery of novel medications is only one aspect of medicinal plant research. Natural goods, whether as standardized plant extracts, offer limitless possibilities for innovative medicine development. [1]. This field has been growing and currently encompasses a wide range of topics such as power bargaining based on medicinal plant expertise. [2]. Plants are given distinct features and properties by these phytoconstituents. As a result, determining various biological activities of plants would be aided by analyzing these elements. Detecting Phyto-components requires simple, cost-effective, and quick assays. Spectroscopic (UV-Vis, FTIR) approaches, as well as conventional procedures, can be employed in this context. [3]
In plants, FTIR provides for the examination of a significant quantity of compositional and operational data. Furthermore, FTIR spectroscopy is a fixed time-saving approach for identification and characterization. [4]. UV-visible spectrophotometry (UV-Vis) is concerned

with photon spectroscopy in the UV-visible region. Light in the visible wavelengths or nearby ranges is used in UV-visible spectroscopy. [5].
Tephrospurpurea Linn. (Fabaceae) is a polymorphic, heavily branched, suberect perennial herb that grows 30-60 cm tall and reaches an altitude of 1,850 m in the Himalayas. Wild indigo, Sarphonk, and Sharpunkha are all names for this plant. [5]
Because previous phytochemical screening revealed that the plant contains coumarins, flavonoids, rotenoids, flavanones, iso flavanones, and quercetins, we tested the roots of Tephrosia purpurea for antioxidant activity in vitro. Tephrosia Malayalam: Purple tephrosia, Wild indigo, Fish Poison, Tephrosia Tephrosin, which paralyzes fish, is found in the leaves and seeds, and is used as a fish poison. Because a decoction of the roots is used to treat rheumatism, asthma, and urinary diseases, we tested antioxidant activity in the roots of Tephrosia purpurea for in vitro study. [7]

2. MATERIAL AND METHODS

2.1 Materials
Methanol (analytical grade), UV- visible double beam Spectrophotometer (Systronics model no.2201), Centrifuge machine (REMI RM-12C), and FTIR

2.2. Collection & Authentication of Plant:
Tephrosia purpurea roots were collected from the Lawari/ Umari village, Bhandara District, and the specimen was identified by the department of botany, M. B. Patel College Sakoli. District- Bhandara.

2.3 Extraction of Material:
The dried root material (250 g/500ml) was extracted successively with methanol using the Soxhlet device at 40-55°C for 8-10 hrs to get the extract of Phyto compounds. [10]. Then the solvent was allowed to evaporate and the extract was used for the phytochemical analysis. [8]

2.4. Phytochemical screening:
The occurrence of phytochemical elements such as alkaloid, carbohydrate, flavonoid, glycoside, tannin, steroid, and saponin in the freshly obtained crude methanolic root extract T. purpurea was qualitatively examined. Using normal techniques, these were identified by distinctive color changes. [11]

2.5. UV-VIS and FTIR spectrophotometer analysis:
Using a high-pressure vacuum pump, the methanolic extracts were centrifuged at 3000 rpm for 10 minutes before being filtered using Whatman No. 1 filter paper. Using the same solvent, dilute the sample to 1:10. The extract was scanned with a UV-visible double beam spectrophotometer at wavelengths ranging from 200 to 900 nm, and the characteristic peaks were observed. For the spectrum conformation, each study was performed three times.[9]

2.6. Determination of Antioxidant Potential:
T. purpurea has shown the antioxidant activity due to the presence of various physiologically active chemicals. In DMBA (7,12-dimethyl benz(a)anthracene) painted rats, an ethanolic extract of this plant exhibited potential against lipid peroxidation as well as increased

antioxidant capacity (Kavitha et al.,2006). [12] T. purpurea leaves have antioxidant properties. Its ethanolic and ethyl acetate extracts were tested for CCl4 (Carbon tetrachloride) induced lipid and superoxide production, with the ethyl acetate extract showing increased antioxidant activity [13]. With oxidative stress and xanthine oxidase activity, T. purpure root extract showed free radical scavenging action (Nile et al., 2011). [14] In the DPPH free radical experiment, the aqueous extract of the whole plant has potential for free radical scavenging activities (De Smet, 1998).[15]

2.6.1. Reducing Power Assay:

Oyaizu's method was used to determine the reduction power of methanolic extracts of tobacco products. [16]. Tobacco was combined with phosphate buffer (2.5 ml) and potassium ferricyanide at varied concentrations in methanol (2.5 ml). This mixture was maintained in a water bath at 50°C for 20 minutes. After cooling, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 minutes as needed. 2.5 mL upper layer solution was combined with 2.5 mL distilled water and 0.5 mL freshly produced ferric chloride solution. The blank was made in the same way as the sample but without the sample. At 700 nm, the absorbance was measured. As a control, various amounts of ascorbic acid were utilized. Increased absorbance in the reaction mixture shows that the reducing power has increased.[17]

2.6.2. Phosphomolybdenum Reduction Assay:

The total antioxidant capacity of the methanol extract was determined using the Phosphomolybdenum reduction test technique, as published by Prieto et al. [18]. The assay is based on the methanol extract's reduction of Mo (VI) to Mo (V) and the subsequent formation of a green phosphate/ Mo (V) complex at an acid pH. 1 ml of extract at varying concentrations (100-300ug/ml) was combined with 1 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 95°C for 90 minutes. A UV-visible spectrophotometer was used to measure the absorbance of the reaction mixture at 765nm. The reference standard was ascorbic acid.[17]

2.6.3. Iron Chelating Assay:

The reaction mixture of 1 mL phenanthroline, 2 mL ferric chloride solution, and 2 mL extract at varying concentrations (50-200 g/ml) in a final volume of 5 mL was incubated for 10 minutes at room temperature, and the absorbance was measured at 510 nm. Instead of extract, standard medication ascorbic acid was used, and absorbance was equated to a total reduction of all ferric ions of 100 percent. Blank was completed without the use of any kind of medication. [18].

% Chelating activity= {1-absorbance (T)/ Absorbance (B)} × 100

Where Absorbance (T): Absorbance of test Absorbance (B): absorbance of Control

2.6.4. Nitric Oxide Scavenging Activity:

Griess' reagent was used to assess nitric oxide scavenging activity. The mixture was incubated at 25°C for 150 minutes with 2 ml of 10 mM sodium nitroprusside in standard phosphate buffer (pH 7.4) and 0.5 ml of extract at varying concentrations (100-400ug/ml). The incubated solution was combined with 1 mL of sulfanilic acid reagent (0.33 percent in 20% glacial acetic acid) and incubated for 5 minutes at room temperature 25°C. Finally, 1 mL of Napthylethylene diamine dihydrochloride (0.1 percent v/v) was added and incubated for 30 minutes at room temperature. A UV-visible spectrophotometer was used to detect the absorbance at 540 nm. [18]

. % Nitric oxide inhibitor activity= (Ao –As/ Ao) × 100

Where, Ao= Absorbance of control

As = absorbance in the presence of the extract.

3. RESULTS AND DISCUSSION

This section should include data obtained from the research and analysed statistically as described in the methods section. This section must include a description of any results that are presented in tabular or graphical form. The results should be discussed in relation to current understanding of the scientific problems being investigated in the field.

The phytochemical screening showed positive results for triterpenes/steroids, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, and phenolic acids.

The qualitative UV-VIS spectrum profile revealed peaks at 342.8 nm, 373.6 nm, 384.8 nm, 433.8 and 459.0 with absorption values of 2.322, 2.297, 2.336, 2.345, and 2.330, respectively (Fig 1).

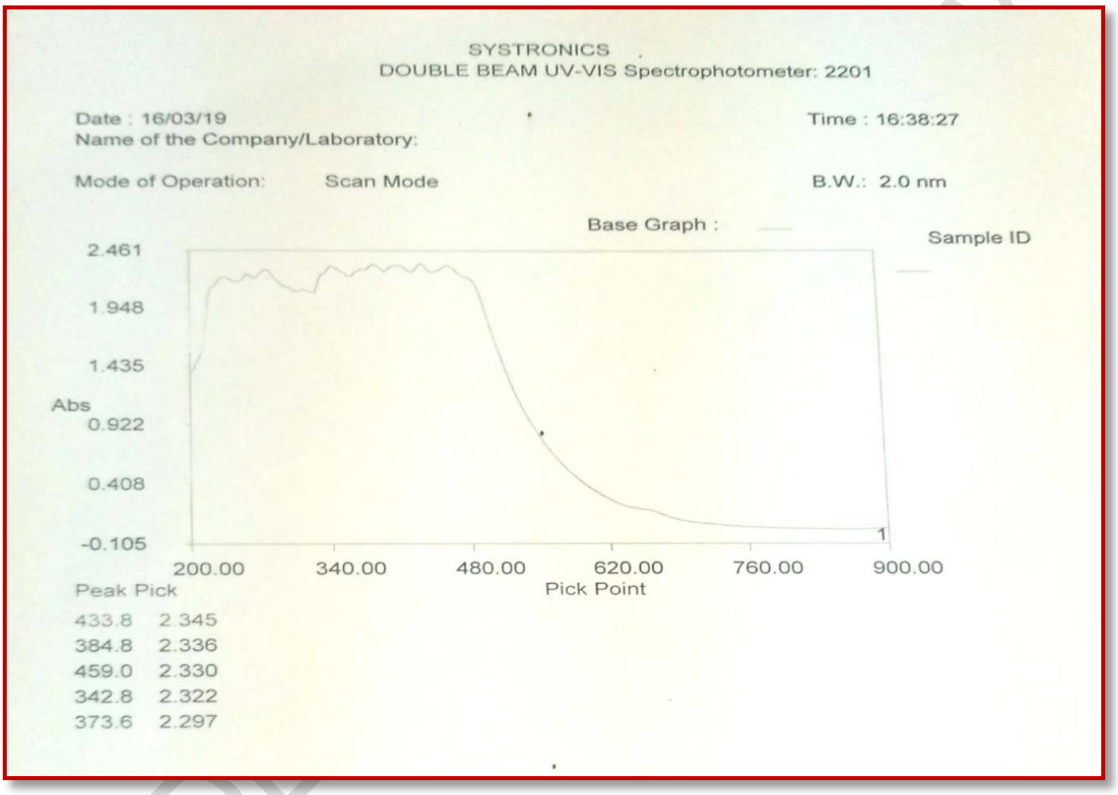

Figure 1. **UV-VIS spectrophotometer analysis:**

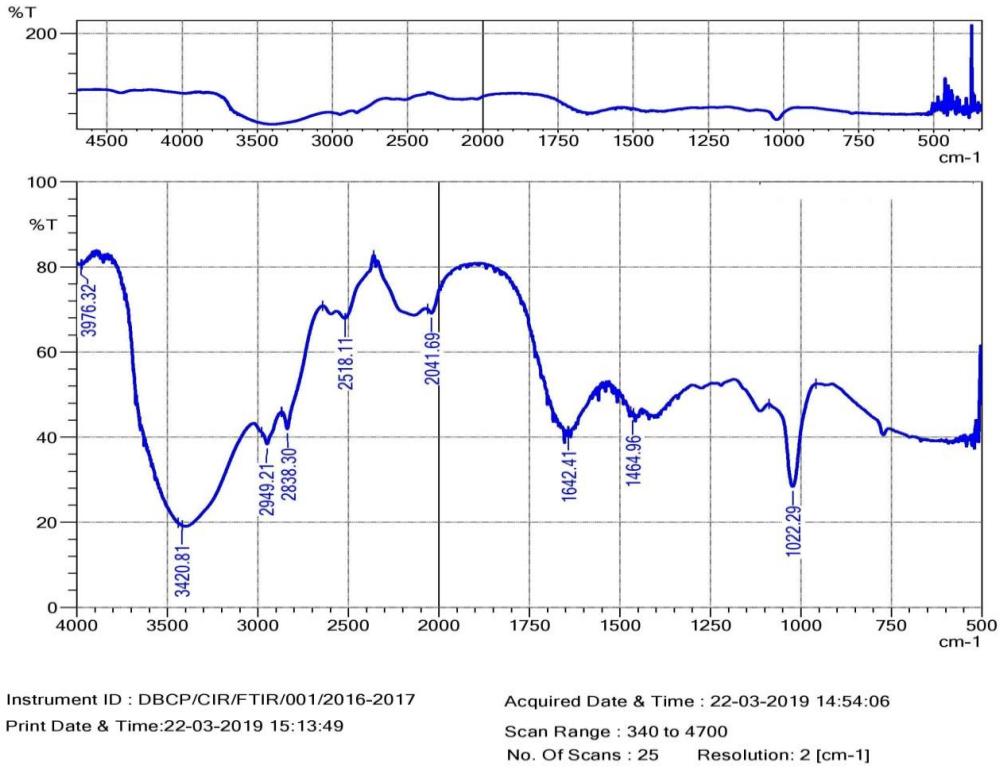

Figure 2. **FTIR spectrophotometer analysis:**

2.6.1. Reducing Power Assay:

The results of reducing power assay the for the various samples was found to be 0.070, 0.0722 and 0.121 compare to the standard solution of Ascorbic acid for 100%, 200% and 300% respectively. The obtained values were found within the limits. (0.048). (Table 1) and Fig 3.

The propose method reducing power assay is the good and accurate for the Phytochemical study of Methanolic Extract of Tephrosia Purpuria Linn Root

Table 1. **Reducing Power Assay**

Concentration	S1	S2	S3	S4	Avg	AS
100	0.117	0.06	0.083	0.021	0.070	0.048
200	0.105	0.05	0.107	0.027	0.0722	0.166
300	0.145	0.135	0.112	0.094	0.121	0.28

S: Sample, AS: Ascorbic acid

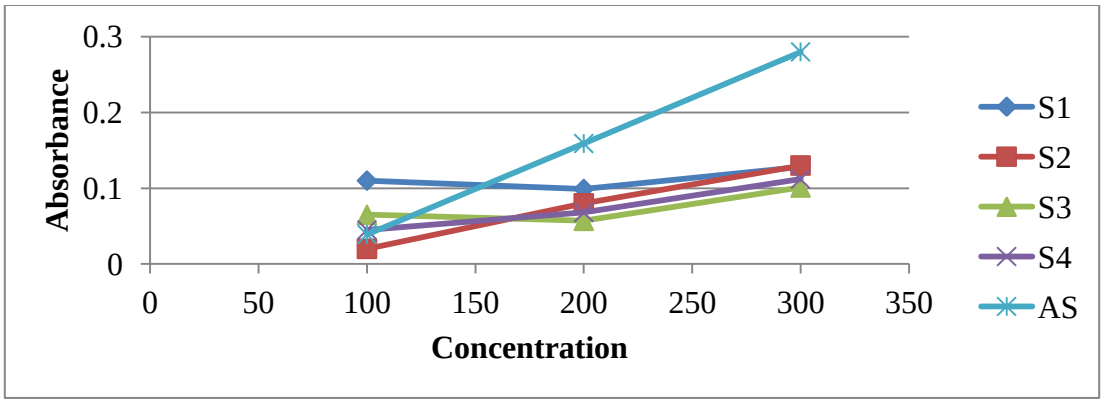

Figure 3. Reducing Power Assay:

2.6.2. Phosphomolybdate Reduction Assay:

The results of **Phosphomolybdate assay** for the various samples was found to be 0.226, 0.414 and 0.543 compare to the standard solution of Ascorbic acid for 100%, 200% and 300% respectively. The found values was obtained within the limits. (Table 2) and (Fig 4)
Phosphomolybdate assay method is good for phytochemical study of Methanolic Extract of Tephrosia Purpuria Linn Root, because the absorbance of sample is accurate comparing with standard ascorbic acid solution.

Table 2: Phosphomolybdate assay

Concentration	S1	S2	S3	S4	Avg	AS
100	0.251	0.257	0.162	0.235	0.226	0.607
200	0.411	0.409	0.376	0.463	0.414	1.15
300	0.571	0.588	0.423	0.593	0.543	1.676

S: Sample, AS: Ascorbic acid

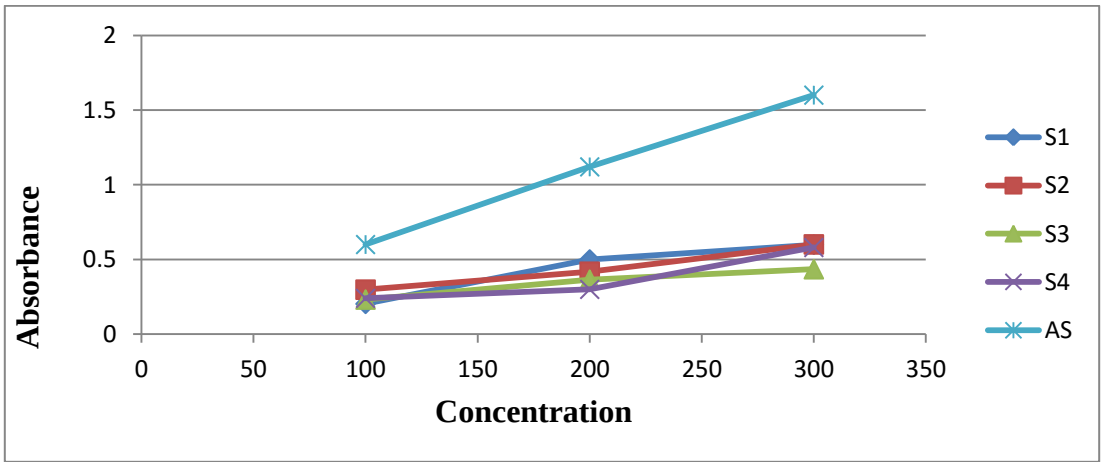

Figure 4. Phosphomolybdate Assay

2.6.3. Iron Chelating Assay:

The results of **Iron Chelating Activity Assay** for the various samples were found to be 0.58, 0.57, 0.59and 0.59 compare to the standard solution of Ascorbic acid for 50%,100%, 200% and 300% respectively. (Table 3) and (Fig 5)

Iron chelating assay giving accurate result of sample comparing with standard solution of ascorbic acid, this method is good and accurate for the phytochemical study of Methanolic Extract of Tephrosia Purpuria Linn Root.

Table 3: Iron Chelating Activity Assay

Concentration	S1	S2	S3	S4	Avg	AS
50	0.55	0.55	0.67	0.57	0.58	0.57
100	0.56	0.55	0.62	0.58	0.57	0.56
150	0.57	0.57	0.64	0.58	0.59	0.54
200	0.58	0.58	0.61	0.59	0.59	0.53

S: Sample, AS: Ascorbic acid.

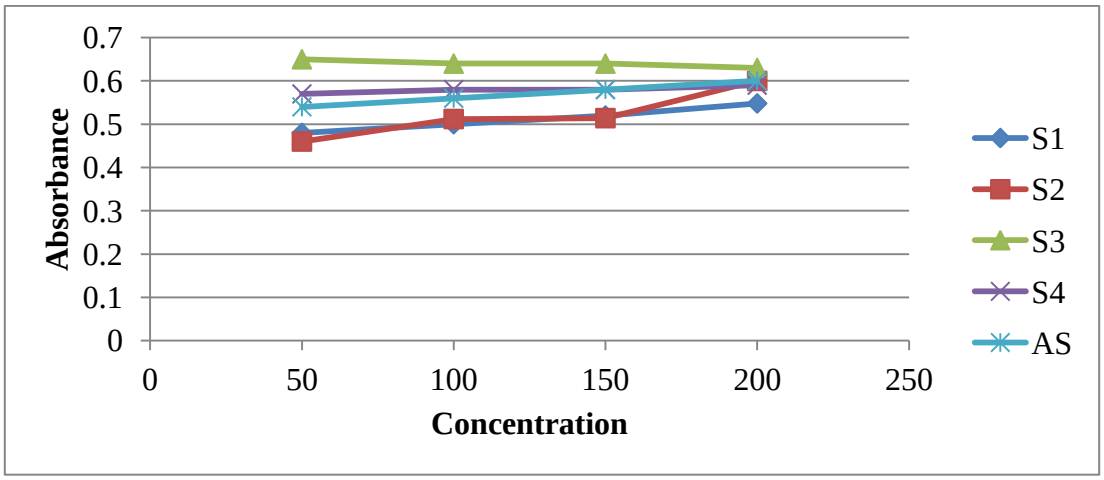

Figure 5. Iron Chelating Activity Assay

2.6.4. Nitric Oxide Scavenging Activity:

The results of **Nitric Oxide Scavenging Activity** for the various samples were found to be 2.47, 4.325, 4.588 and 7.957compare to the standard solution of Ascorbic acid for 100%, 200%,300% and 400% respectively. The found values was obtained within the limits. (Table 4) and (Fig 6)

This method is good for the phytochemical study of Methanolic Extract of Tephrosia Purpuria Linn Root because the absorbance of sample is accurate comparing with standard ascorbic acid solution.

Table 4: Nitric Oxide Scavenging Activity

Concentration	S1	S2	S3	S4	Avg	AS
100	5.02	2.79	1.11	0.55	2.47	1.98
200	7.26	2.79	6.14	1.11	4.325	5.36
300	7.26	3.35	7.26	1.67	4.588	9.25
400	15.64	3.91	8.93	3.35	7.957	12.6

S: Sample, AS: Ascorbic acid.

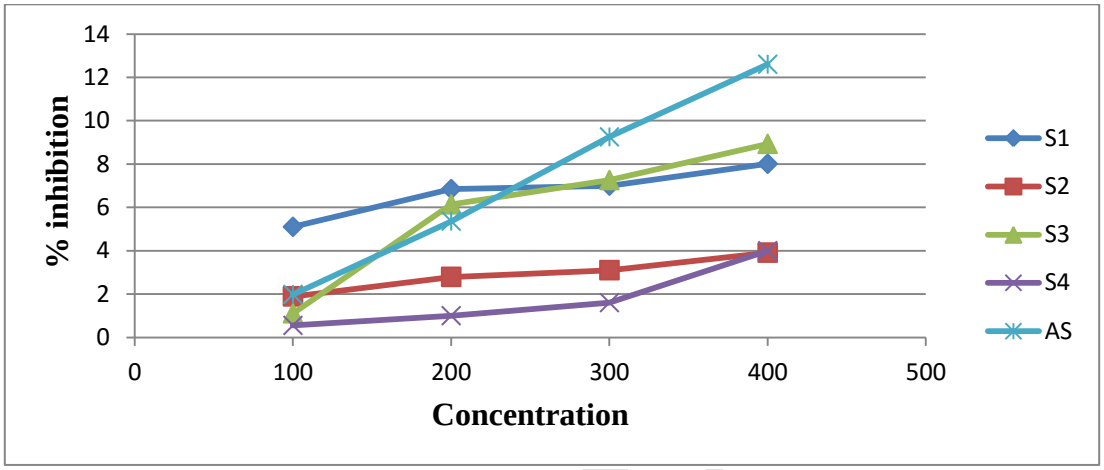

Figure 6. Nitric Oxide Scavenging Activity

CONCLUSION

From the results of the study, the extract of Tephrosia purpuria was found to be having very good antioxidant activity in in-vitro evaluation by different methods. The plant extract of T. purpuria showed presence of phyto-constituents like flavonides, saponines and tannins in photochemical screening which might be responsible for strong antioxidant activity.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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