



### RESEARCH ARTICLE

## PHYTOHEMAGGLUTINATION ASSAY OF CAMELLIA SINENSIS AND CAMELLIA ASSAMICA EXTRACTSON MEMBRANE RECEPTORS OF ERYTHROCYTE SURFACES

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### Abstract

The objective of this study is a comparative analysis of the changes observed on the red blood cell antigens which differ in a short glycoprotein chain difference, by the effects of a *Camellia sinensis* and *Camellia assamica* extracts. The hematological effect of the extract was prepared and evaluated using blood group analysis on hematological indices. Agglutination was clearly observed initially for 5 min for all the eight blood types. Specifically, for the O+ blood group there is a time lag for agglutination to take place for the incubation period of 15 min for *C. sinensis*, while agglutination occurred after 10 minutes of adding blood group antibodies with *C. assamica*. These results, as well as the possibility of adapting this method to a fully automated system, could be an important contribution to the field of immunohematology.

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### Introduction:-

ABO and RhD variants are the most studied blood group in India.<sup>[3]</sup> Progress is being made towards modification of red blood cell surface antigens from A and/or B to blood group O.<sup>[6]</sup> In present study, we have been undertaken to explore the effect of *Camellia sinensis* and *Camellia assamica* extract towards modification of red blood cell surface antigens.<sup>[19]</sup> Plants are universally recognized as a vital part of the world's natural heritage and up to 80% of the population rely on plants for their primary healthcare.<sup>[5, 21]</sup> Varieties of medicinal plants are recognized as a source of natural antioxidants that can protect from oxidative stress,<sup>[7]</sup> thus playing an important role in chemoprevention of diseases. Each of these plants has some effect on the endothelium,<sup>[9]</sup> blood cells,<sup>[11]</sup> angiogenesis,<sup>[2, 4]</sup> cellular proliferation,<sup>[13]</sup> vascular dynamics<sup>[18]</sup> and cell mediators.<sup>[16, 17]</sup> The chemical composition of the leaves indicates the presence of polyphenols, which have antioxidant properties. Erythrocytic membrane instability is possibly a major factor as has been earlier reported with ethanol and chloroquine for the cytotoxicity of these plant extracts.<sup>[20]</sup> Research findings suggest that anthocyanin extracts in a plant play a role in both stabilizing the red blood cell membrane and inhibiting polymerization of hemoglobin.<sup>[11]</sup> This provides a possible molecular basis for earlier reports on the anti-sickling properties of anthocyanins from some plants and their use in the management of disease by traditional healers.<sup>[15]</sup>

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## Material and Methods:-

### Preparation of Smoothie:

Extraction was carried out by grinding seeds of the plant with a pestle and mortar in the presence of liquid nitrogen and or made into smoothie<sup>[20]</sup>. The material was transferred to a vial and 500µl of lysis buffer (HiMedia) was added and the sample was vortexed. Later, the suspension was mixed for one hour at 37°C and filtered. The filtrate was spin at 14,000 rpm (Thermo, MicroCL 21 Microcentrifuge) in cold conditions for 10 min and supernatant was removed and stored at 4°C until further analysis.

### Blood sample collection and processing:

Blood samples were obtained from eight healthy individuals (males and females) aged 18 to 20 years. Informed consent was obtained from everyone before collection of blood, and the procedures followed were in accordance with the ethical recommendations (23/IEC/IG/2017) of our institution<sup>14</sup>. The samples were kept at 4°C and divided into groups, based on blood typing, using ABO diagnostic kit (Span Diagnostics).<sup>[10]</sup>

### Chemical analysis of extract:

The alcoholic extract showed the presence of flavonoid, phenolics, glycoside, saponins, alkaloid, and polysaccharide when subjected to qualitative chemical tests.<sup>[8]</sup> The total phenolic contents estimated were done according to standard published method FolinCiocalteu reagent. Aluminum chloride colorimetric method was used for flavonoid determination.<sup>[12]</sup>

### Determination of blood type conversion:

Briefly, whole-blood cells from A, B, and O blood donated from healthy volunteers were loaded on agglutination plate. 5µL of the Anti-A or Anti-B or Anti-D antibodies were added to human A- or B-type whole-blood cells on a white acryl plate, and the agglutination reaction was determined within 30s of its onset.<sup>[14]</sup>

## Results:-

### Blood sample collection and processing:

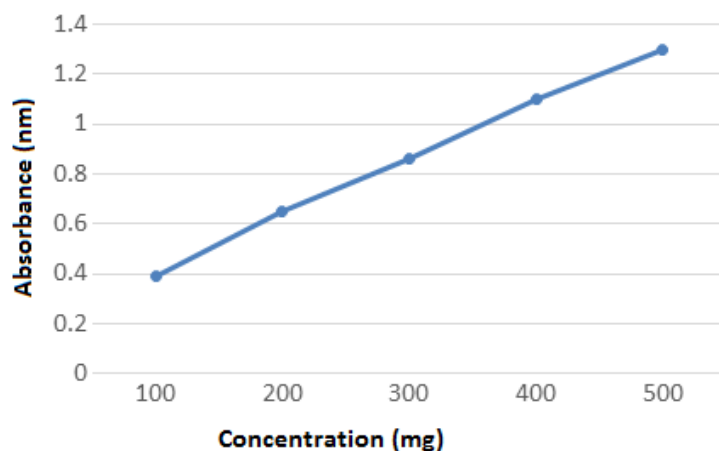
All the samples collected from the informed consent have been processed and analyzed for its type. The blood was collected into a vacuum glass tube or heparin tubes used as anticoagulant for the determination of blood group. All eight samples were processed and analyzed using ABO diagnostic kit (Span) and represented in table 1.

**Table1:-** ABO blood typing of various samples collected from volunteers.

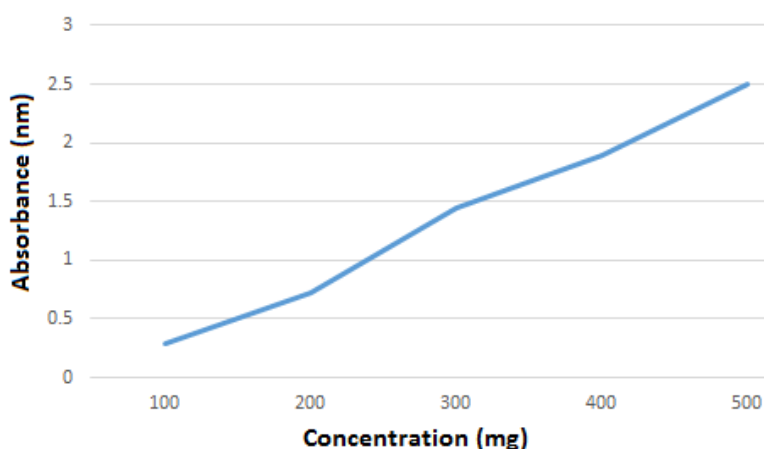
Sample ID	Age	Blood type
KLEF01	21	A+
KLEF02	20	A-
KLEF03	19	B+
KLEF04	18	B-
KLEF05	21	AB+
KLEF06	20	AB-
KLEF07	18	O+
KLEF08	20	O-

### Chemical analysis of extract:

Total phenolic and flavonoid contents were found about 13% and 3% respectively in the extract of *Camellia sinensis* and *Camellia assamica*. Total phenolic content was determined according to Folin-Ciocalteu method. The concentration of phenolic compounds in test samples was calculated from the standard gallic acid curve. The standards graph of gallic acid and quercetin for total phenolic and flavonoid contents are shown in figures 1 and 2 respectively.



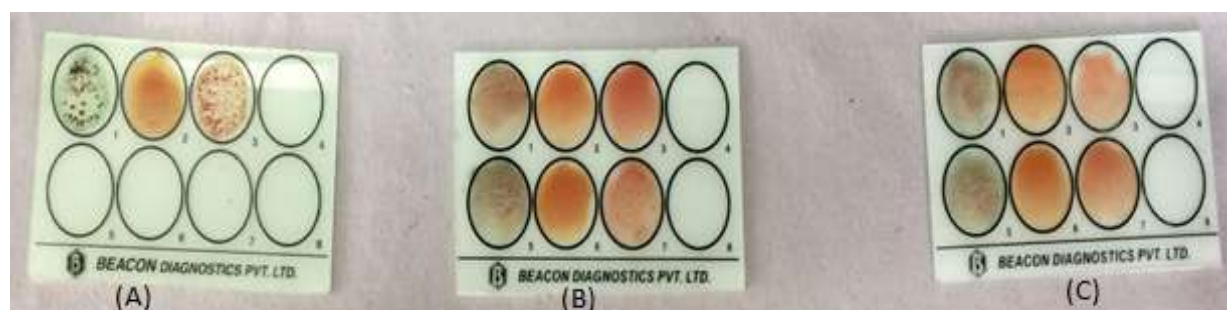
**Figure 1:-** Standard curve of gallic acid for total phenolic contents.



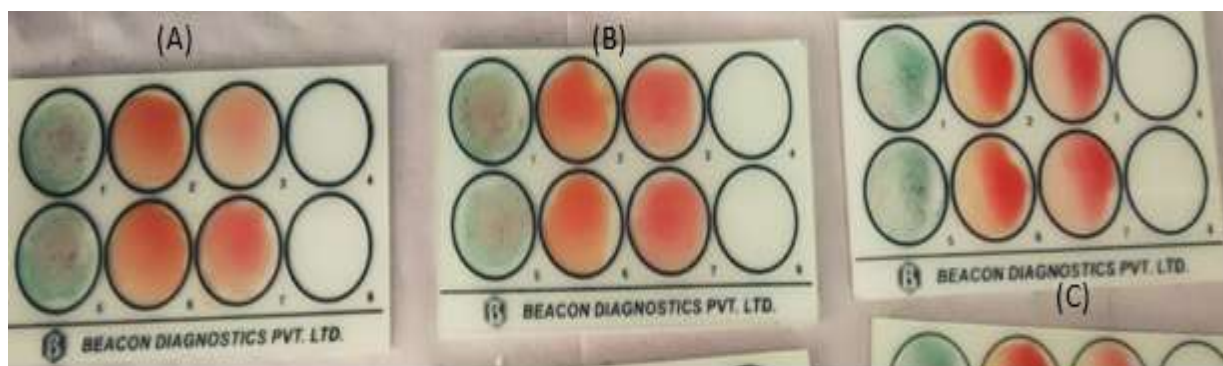
**Figure 2:-** Standard curve of quercetin for total flavonoid contents.

#### Determination of blood type conversion:

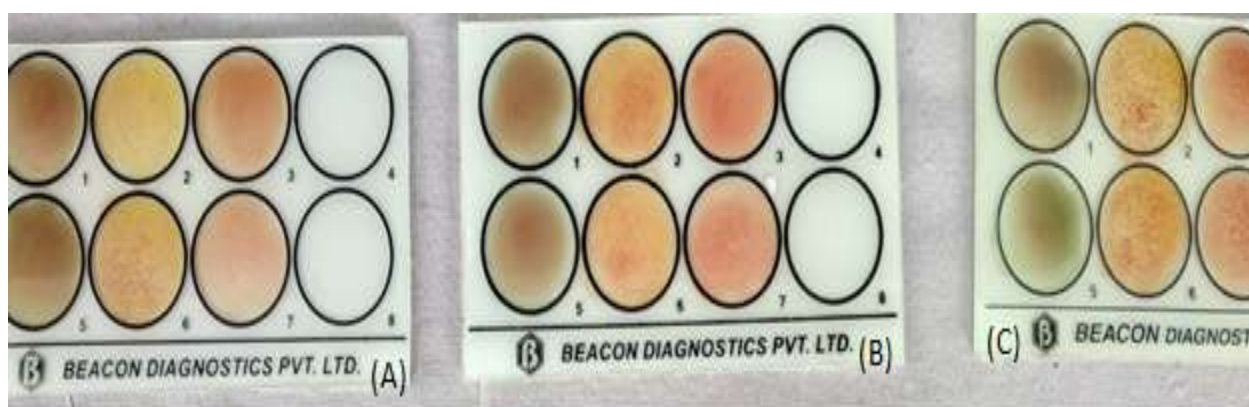
Analysis was run in duplicates ( $n=2$ ) to verify the difference in the effect of extracts on blood samples. First, it is observed that there was no effect of different extracts of processing on the enzyme activities on blood group. Later for the increase in incubation time, the agglutination was gradually decreased. Specifically, for the O+ blood group there is a time lag for agglutination to take place for the incubation period for 15 min for *Camellia sinensis*, while agglutination occurred after 10 minutes of adding blood group antibodies with *Camellia assamica* (Figure 3-10).



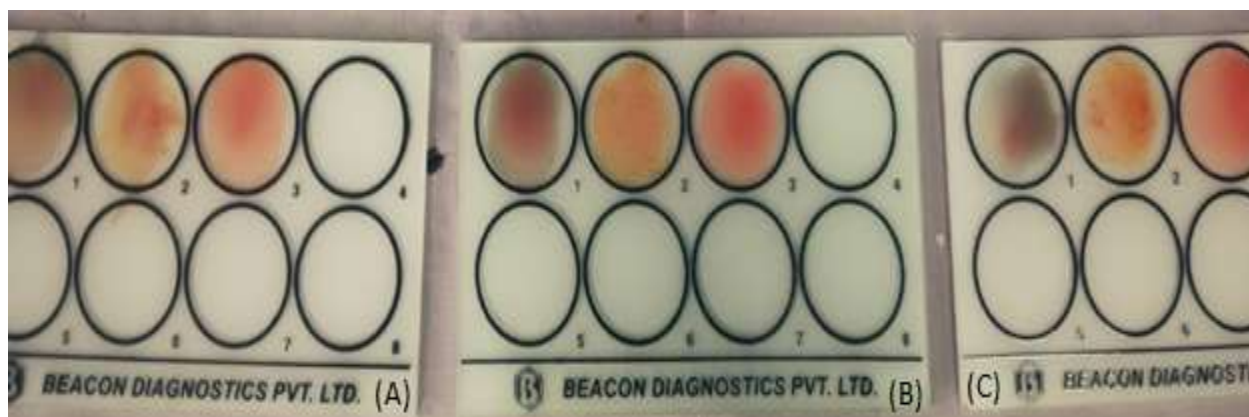
**Figure 3:-** Haemagglutination assay of RBCs by ABO slide method for blood type A+. (A) Agglutination was observed in zone 1 and 3 indicating the test as positive for blood type A+ (positive control test) (B) Agglutination of A+ blood type was hampered after incubating with *Camellia sinensis*, because zones were dark and uniform in color, indicating no agglutination. (C) Test is specific for *Camellia assamica* however, resulted in low level agglutination.



**Figure 4:-** Haemagglutination assay of RBCs by ABO slide method for blood type A-. (A) Agglutination was observed in zone 1 indicating the test as positive for blood type A- (positive control test) (B) Test is specific for *Camellia sinensis*, resulted in normal agglutination. (C) Test is specific for *Camellia assamica*, resulted in normal agglutination.

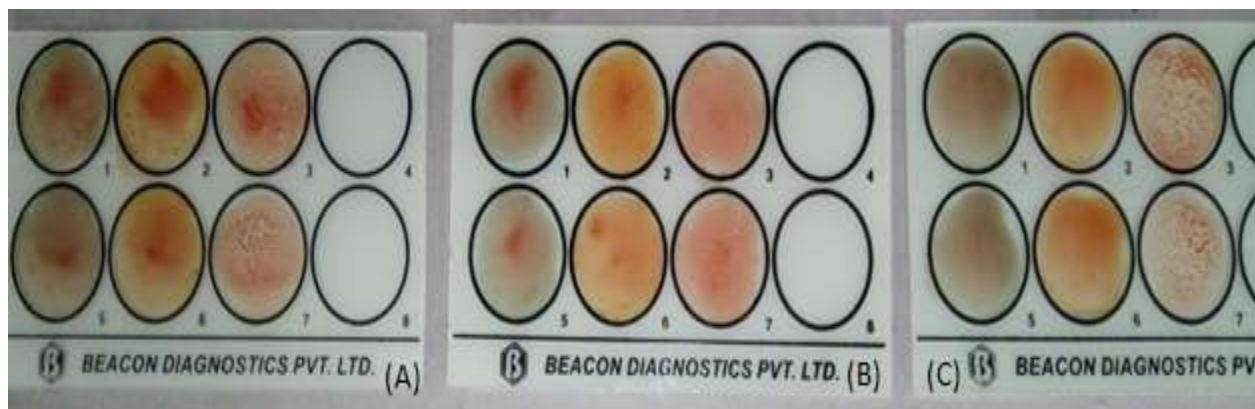


**Figure 5:-** Haemagglutination assay of RBCs by ABO slide method for blood type B+. (A) Agglutination was observed in zone 2 and 3 indicating the test as positive for blood type B+ (positive control test) (B) Test is specific for *Camellia sinensis*, resulted in normal agglutination. (C) Test is specific for *Camellia assamica*, resulted in high level agglutination.

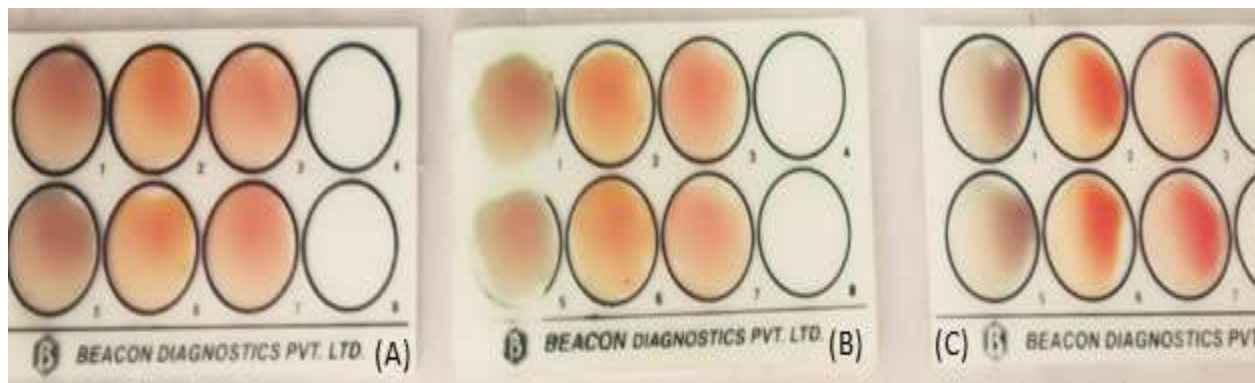


**Figure 6:-** Haemagglutination assay of RBCs by ABO slide method for blood type B-. (A) Agglutination was observed in zone 2 indicating the test as positive for blood type B- (positive control test) (B) Test is specific for *Camellia sinensis*, resulted in normal agglutination. (C) Test is specific for *Camellia assamica*, resulted in normal agglutination.





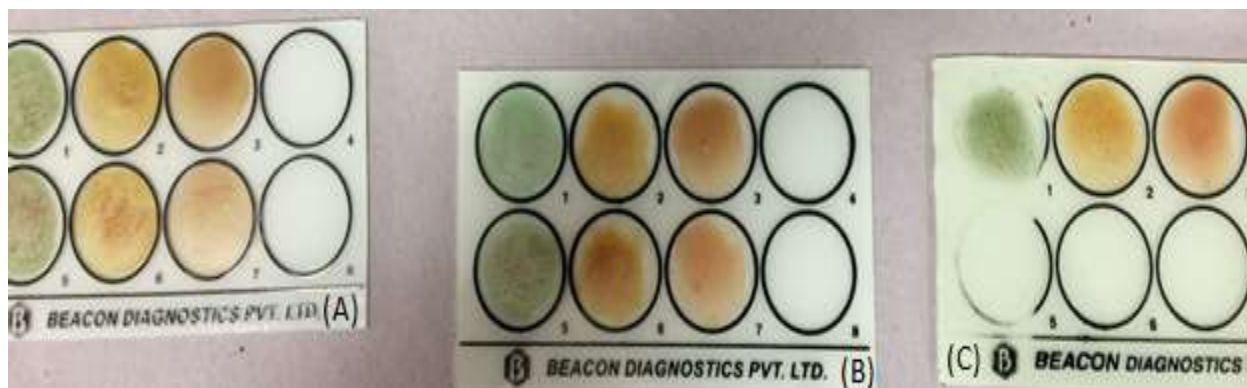
**Figure 7:-** Haemagglutination assay of RBCs by ABO slide method for blood type O+. (A) Agglutination was observed in zone 3 indicating the test as positive for blood type O+(positive control test) (B) Test is specific for *Camellia sinensis*, resulted in low level agglutination. (C) Test is specific for *Camellia assamica*, resulted in high level agglutination.



**Figure 8:-** Haemagglutination assay of RBCs by ABO slide method for blood type O-. (A) Agglutination was not observed in either zone 1, 2 or 3 indicating the test as positive for blood type O- (positive control test) (B) Test is specific for *Camellia sinensis*, resulted in nil agglutination. (C) Test is specific for *Camellia assamica*, resulted in nil agglutination.



**Figure 9:-** Hemagglutination assay of RBCs by ABO slide method for blood type AB+. (A) Agglutination was observed in zone 1, 2 and 3 indicating the test as positive for blood type AB+ (positive control test) (B) Test is specific for *Camellia sinensis*, resulted in normal agglutination. (C) Test is specific for *Camellia assamica*, resulted in high level agglutination.



**Figure 10:-** Hemagglutination assay of RBCs by ABO slide method for blood type AB-. (A) Agglutination was observed in zone 1, 2 and 3 indicating the test as positive for blood type AB- (positive control test) (B) Test is specific for *Camellia sinensis*, resulted in nil agglutination. (C) Test is specific for *Camellia assamica*, resulted in nil agglutination.

### Discussion:-

The impact of the extracts on sample processing in the laboratory was evaluated for agglutination assays for ABO and D (Rh factor). For all the eight blood group samples agglutination initially for 30s was clearly seen for *Camellia sinensis* and *Camellia assamica* at 0.05 mg/mL. Haemagglutination assay of RBCs by ABO slide method for all eight blood types were evaluated and found to be effective for blood type B+ compared to other types. Although further work has to be extensively carried out to determine the effect of the coffee bean extracts at high incubation periods and also for high concentration levels.

### Conclusion:-

Traditional blood typing methods are simple, sensitive and reliable, yet time consuming and labor intensive; moreover, the cost of blood group-specific antibodies is quite high. Hence an immediate approach for the modification of red blood cell antigens is needed for a compatible blood transfusion. Here, we have described a comparative study for effect of the extracts for haemagglutination detection on all types of blood groups. The difference between the extracts were evaluated and estimated to determine the modification of RBC surface antigen. The agglutinated samples elicited changes between 10% and 35% in the O+ and B+, while the non-agglutinated samples induced a maximum change of 7.5% in all other six blood types.

### Conflict of interest:

The authors declare no conflict of interest.

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